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Food poisoning.

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FOOD POISONING

FOOD POISONING

Revised Edition

By

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THE UNIVERSITY OF CHICAGO PRESS

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F8,3;F2
N43

THE UNIVERSITY OF CHICAGO PRESS, CHICAGO 37
Cambridge University Press, London, N.W. 1, England
W. J. Gage & Co., Limited, Toronto 2B, Canada

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CFTRI-MYSORE
483
Food poisoning

This book is dedicated to
the memory of my colleague and friend
WILLIAM E. CARY

PREFACE TO THE SECOND EDITION

THE useful purpose which this little book has served during and after World War II has been gratifying beyond expectation to its author. The problems of processing and distributing food during the war added to our knowledge of food poisoning; hence an attempt has been made to bring the subject matter up to date, and, in so doing, the author has indebted himself to many friends and colleagues. Among those to whom reference is not made in the text are Mr. Hiroshi Sugiyama for his help in revising the chapter on botulism, Dr. Michael Surgalla for assistance with the chapter on staphylococcus food poisoning, Dr. Elizabeth Verder and Dr. Morris D. Schneider for their help in revising the chapter on *Salmonella*, and Dr. Charles Niven, Jr., for his assistance in revising the chapter on α-type streptococci in relation to food poisoning. Dr. K. Eileen Hite has read many portions of the revision and offered helpful criticism. Miss Joan Kadavy has been especially helpful in checking the Bibliography. The author is particularly indebted to his secretary, Miss Shirley Ann Kenney, whose tireless efforts have made this revision possible.

G. M. DACK

PREFACE TO THE FIRST EDITION

THE term "food poisoning" is, of necessity, vague. Properly included in this category are illnesses resulting from ingestion of food containing certain inorganic chemicals, poisons derived from animals and plants, and toxic products or infections caused by several species of bacteria. Exact characterization of these disorders, produced by such a diverse group of inciting agents, is impossible because they do not present a uniform syndrome and are therefore variously classified by different authors.

Perhaps the most characteristic feature of an outbreak of food poisoning is the explosive nature of the illness, which is generally characterized by gastrointestinal upsets. There are, however, important exceptions, such as, for example, botulism, in which the gastrointestinal symptoms are generally a minor feature of the disease.

In the present book I have included the following causative agents: chemicals, poisonous plants and animals, bacteria and their products, viruses, protozoans, helminths, and a miscellaneous group of illnesses, the causes of which are unknown. Of this list, the bacteria which form toxins in food before ingestion are discussed at greatest length. Botulism and staphylococcus food poisoning, which are induced by toxins pre-formed in the food, represent the more typical diseases in this classification. The gastrointestinal upsets associated with the *Salmonella* infections should be considered more strictly as food-borne infections, although they are universally classified as food poisoning.

It is a pleasure to acknowledge my indebtedness to many friends and associates for invaluable aid in my work in this field during the last twenty years. I am indebted to Professor F. W. Tanner, under whose direction my interest in food poisoning was aroused; to the late Professor E. O. Jordan, with whom I was associated as student and colleague; and to other associates with whom I worked at various times, in particular to Professor W. A. Starin, Drs. W. E. Cary, Willard Wood, James Gibbard, Oram C. Woolpert, Ellen Davison, and Milton Segalove.

I am indebted to Professors W. H. Taliaferro and R. W. Harrison and to Dr. Ellen Davison for help in preparing this book, and I am especially indebted to Mrs. W. H. Taliaferro for revising the manuscript.

G. M. Dack

TABLE OF CONTENTS

I. INTRODUCTION 1

II. CHEMICAL POISONING IN FOOD 12

III. POISONOUS PLANTS AND ANIMALS 26

IV. BOTULISM 42

V. STAPHYLOCOCCUS FOOD POISONING 80

VI. SALMONELLA 119

VII. ALPHA-TYPE STREPTOCOCCI (*Streptococcus faecalis*) IN RELATION TO FOOD POISONING 146

VIII. SIGNIFICANCE OF OTHER BACTERIA IN FOOD POISONING . . . 155

IX. INFECTIONS TO BE DIFFERENTIATED FROM FOOD POISONING . . 162

AUTHOR INDEX 173

SUBJECT INDEX 179

CHAPTER I

Introduction

DURING the past century great changes have taken place in man's mode of life. This has brought about new methods of processing and preparing foods. The modern age of invention has brought with it specialization in industry, and the individual is no longer called upon to produce his own food. With modern transportation and refrigeration, fresh fruits and vegetables are grown in favorable areas and shipped, either canned or fresh, to distant markets. The same situation applies to the canning or handling of fresh meats. Experience has shown that food-poisoning problems may arise when new procedures are instituted in preparing and storing foods.

Although food poisoning may result from a diverse group of inciting agents, there is still a tendency to disguise the causative agent under the blanket term of "ptomaine" poisoning. This term has been in common use since its introduction in 1870 by Selmi, the Italian toxicologist. It was taken from the word *ptoma*, meaning "corpse." Since there is neither a specific entity nor a group of substances which can properly be called "ptomaine," the term is unscientific and meaningless.

There is a popular tendency to associate food poisoning with putrefaction. It is now known that putrefaction in a food does not necessarily or even ordinarily give rise to the toxic substances involved in food poisoning. An example of a wholesome food containing putrefactive bacteria and their products is Limburger cheese. Other examples (1)* are rotten seal meat and fish eaten by the Eskimos in Greenland, rotten fish by the

---

* All references will be found at the end of each chapter.

Samoyedes, rotten eggs by the Chinese. Many other peoples of the world consider rotten meat a delicacy. Another case in point is food contaminated with *Clostridium sporogenes*, one of the most putrefactive of bacteria. Cultures of this bacterium have been repeatedly fed to experimental animals without producing any evidence of illness. Furthermore, these cultures have been injected intraperitoneally and intravenously without causing ill effects. As a matter of fact, cultures of *Cl. sporogenes* were used in World War I to treat suppurative war wounds, with reported beneficial effects (2).

INVESTIGATION OF FOOD-POISONING OUTBREAKS

Small outbreaks of gastrointestinal upsets occurring in the family are usually not reported. In general, no recognition is given the event, and the family physician is seldom summoned. When several people are affected, however, particularly when they have attended a banquet or recently eaten at the same public eating establishment, considerable attention is given the episode by health authorities and the press. Illnesses in small family groups due to the fatal type of food poisoning known as "botulism" are usually reported, but this type of food poisoning is relatively uncommon.

Such small family outbreaks as do become known are usually brought to light through complaints made by a member of the family to the grocer or butcher regarding a suspected item of food. In the majority of cases the complaint is carried no further, but reports are sometimes made by the retailer to the manufacturer or producer, and these, in most instances, are investigated. Much time often elapses between the illness and the investigation, thus making it difficult or impossible to determine the causative agent. Frequently, meat is suspected by the family as the cause, since it conforms with the false "ptomaine" notion of food poisoning. Very often, upon investigation, the food item alleged by a member of a family to be responsible for an outbreak is exonerated, and some other item of food or other cause is found.

One of the distinctive characteristics of an outbreak of food poisoning is the sudden illness of several individuals following a specific meal and usually ascribed to a definite item of food served at that meal. Often only one person in such a group develops gastrointestinal symptoms. Some of these single illness cases are actually brought to court for trial. The alleged symptoms are one or more of the following: nausea, vomiting, diarrhea, and abdominal cramping. Careful study of such individuals often reveals one of a large variety of other illnesses to which these symptoms can be attributed. Such illnesses are coronary heart disease, gall-bladder disease, passage of renal calculi, appendicitis, brain tumors, certain forms of intestinal allergy, cerebral hemorrhage, onset of infectious diseases, and functional bowel distress associated with emotional upsets. Local chilling of the abdomen has been reported to give rise to many cases of acute nonspecific diarrhea (3). This was particularly observed in hot climates among military personnel in World War II.

It is important when an outbreak of food poisoning occurs that it be immediately investigated if the true cause is to be established. A delay of hours after vomiting and diarrhea have occurred may mean that chemical poisons have been discarded in the vomitus and excreta. If none of the original food is left, it may be difficult to identify the specific agent. In food poisoning of bacterial origin, a delay of hours may often bring about marked bacterial changes in a perishable product which were not present at the time of consumption. From a legal standpoint, a prompt and intelligent investigation may prevent unnecessary litigation expense and afford the basis of a just settlement if settlement appears warranted. It is generally important to ascertain in so far as possible what items of food had been eaten at previous meals for a period of 48 hours. When a large number of cases occurs within as short a period as 2–3 hours after partaking of food, attention should be centered particularly on the last meal and on any food eaten by all or a majority of the persons afflicted. When the onset of symptoms in different individuals appears over a period of many hours, it is important to know the items of food in the diet for several meals past. If the outbreak involves persons who have shared in a particular meal or eaten at a particular place, it is a good plan to prepare a list of the various items of food served and check these items with persons who ate the meal or who ate at the place in question. Frequently, a single item may be found to be the common denominator for those who are made ill. If this fact is observed, much time may be saved by having a laboratory examination made directly on the incriminated item of food. Usually, not every individual will be made ill when a specific item is involved. Such variables as the amount eaten (dosage) and individual resistance play an important part. Generally, however, the majority of people partaking of the involved food will be affected when such an outbreak occurs. Sometimes psychic disturbances account for symptoms in some individuals who are present during the illness of others but who have not eaten the incriminated food. If too long a time elapses before the investigation is started, the data obtained are less reliable, since most people find it difficult to remember what they have eaten at previous meals or whether they experienced any symptoms at the time in question. An excellent questionnaire has been prepared by individuals concerned with the investigation of food-poisoning outbreaks. These forms are reproduced here.

FORM 1

QUESTIONS FOR PERSONS ALLEGING ILLNESS FROM FOOD

1. What is the name?..Age?..................
2. Address?..
3. Are you employed in any business and, if so, by whom?..........................
   ....
4. Married or single?..
5. How long after eating were you made ill?..
6. Were others made ill?..........................If so, who? (Age of each?)..........
   ....
   ....
7. Did any person eat the same foods without becoming ill?.......................
8. If so, who? (Names and addresses?)...
   ....
   ....
9. What were the first symptoms of your illness and when did they occur?
   ....
   ....
10. Was there pain in the stomach or abdomen?..
    ....

11. Was there vomiting?..................For how long?..................................

12. Was there diarrhea or constipation?..

13. Please tell anything else you remember about your illness and the events leading up to it:..

....

....

....

....

....

....

14. How long did the acute illness last?...

15. Please itemize expenses, if any, resulting from your illness:..........................

....

16. What is the name and address of the doctor who attended you during your illness?..

....

....

17. Is he your regular doctor?............................If not, who is?..................

....

18. When were you last attended by a doctor before this illness and for what cause?...

19. Have you ever undergone an operation and, if so, for what?............................

....

20. Have you had stomach or digestive trouble in the past?.................................

21. If so, of what nature?...

....

22. To what did the doctor attribute the illness?..

....

23. Do you attribute the illness to the eating of any particular food?......................

24. If so, what food?...

....

25. Why?..

26. What foods did you eat at the suspected meal?..

....

27. What was eaten at the four meals preceding the suspected meal? (All foods eaten at each meal, if possible, and also between meals.)...............................

....

....

....

....

28. What foods, if any, did you eat between the suspected meal and the onset of your illness?...

....

29. Did the doctor take the container or any of the suspected food left after the meal?..

30. Did you feed any of the suspected food to dogs, chickens, or other animals?..........

....

If so, were they made sick?..........

31. Have you the container or any portion of the foods which you believe caused the illness?..........

32. If not, what was done with them?..........

....

33. From whom was food purchased and by whom?..........

....

....

34. Date of purchase:..........

35. Give name of brand and of manufacturer or distributor on label:..........

....

36. Did you ask for that particular brand?..........

37. Are you accustomed to buying that particular brand?..........

38. Where was the food left before using?..........

39. Where was the food kept after unwrapping or opening?..........

....

40. State as exactly as possible how the food was prepared and served..........

....

....

41. Did the appearance or odor indicate anything unusual in the food on unwrapping or opening?..........

If so, please give particulars:..........

....

42. On what date and at what meal was the food eaten?..........

....

43. At the time of eating the food, did you notice any peculiarity in the appearance, taste, or odor of it?..........If so, please describe:..........

....

....

Witness:.......... ....

Date:.......... [*Signature*]

FORM 2

QUESTIONS FOR THE ATTENDING PHYSICIAN IN CASE OF ALLEGED ILLNESS

1. Name and address of physician:..........

....

2. Name of patient:..........

3. On what date or dates did you treat this patient?..........

....

4. How long had the patient been ill when you arrived?..........

....

5. Was the onset of the disease sudden or had there been previous illness?
....
6. How many persons were ill of the same type of disease and under your observation?..
7. What were the general symptoms (appearance of patient, continuous or spasmodic pains, nervous, excited, or in coma, vomiting, or diarrhea)?
....
....
8. What was the pulse rate?..
9. Regular or intermittent?..
10. Pulse tension?..
11. Was the temperature persistently above or below normal?..........................
12. What were the highest and lowest points reached?..........................
13. Was a temperature and pulse record kept?..........................
14. What was the number of respirations per minute and character?..........
....
15. Was there any disturbance of vision?..........................
16. What disturbance of the reflexes, if any, was noted?..........................
....
....
17. How long did the attack continue?..........................
18. General remarks:..
....
....
19. Was any bacteriological or chemical examination made of the blood, vomitus, or excreta?..........................
20. What was your diagnosis?..........................
21. If food poisoning, what food do you believe caused the illness?..........
....
22. Was a laboratory examination made of any suspected food?..........
23. If so, what was found?..........................
24. What treatment was followed or prescribed?..........................
....
25. Was any morphine or opiate used to alleviate pain?..........................
26. Was any strychnine or other stimulant used?..........................
27. Do you consider recovery complete?..........................
....
28. Have you a detailed record of the case?..........................
29. Have you attended this patient before?.......... If so, for what ailment?....
....

In case of death:

30. Was an autopsy performed or any post mortem specimens examined?.......
....
31. If so, by whom was such autopsy or examination made?..........................
....

Witness:.. ....

Date:..194..... [*Signature*]

When the investigator has obtained the necessary information, it is generally possible to get some clue as to the nature of the agent involved, so that treatment of the patient may be immediately instituted. Furthermore, if a specific agent is suspected, much time is saved in the laboratory tests. The problem of determining whether the agent is chemical, plant, or animal poison, intoxication with bacterial products, or a food-borne infection is often difficult. The solution of this problem is simplified by a careful field investigation. Reference to Tables 1, 2, and 13 and to the following résumé of some of the general points for differentiating the causative agents will prove helpful in this regard. A more complete account of the specific agents is detailed in subsequent chapters. The final diagnosis in all instances is made in the laboratory.

Chemical poisoning.—In general, chemical poisoning is characterized by a short interval of time elapsing between the swallowing of the poisonous food and the onset of the symptoms. This lapse of time may vary from 10 minutes to 2 hours. The history of the outbreak may suggest the specific chemical. For example, antimony poisoning has occurred from eating foods cooked in cheap, gray-enameled cooking utensils. Cadmium poisoning is usually associated with acid liquids in cadmium-plated metal utensils. Both these chemical poisons give rise to vomiting, cramps, and diarrhea about ½ hour after taking the food in question. Sodium fluoride poisoning should be thought of if this insecticide is used in the kitchen. This chemical is often mistaken for baking powder, soda, or flour. In addition to vomiting, cramps, and diarrhea coming on within a few minutes to 2 hours after eating, paresis of certain groups of muscles may occur and suggest this substance. Acute poisoning due to arsenic, lead, or mercury is not usually associated with foods except where these chemicals have been added with malicious intent. However, lead and arsenic used in sprays and alkaloids should be considered.

Poisonous plants or animals.—The nature of poisoning from plants and animals is varied. Mussel poisoning is suggested if mussels from the Pacific Coast are eaten at certain seasons of the year. Symptoms of respiratory paralysis with the loss of power in the muscles of the extremities and neck would also suggest this poisoning. Favism would be associated with eating the fava bean or being around these plants when they are in blossom. The symptoms are characterized by acute febrile anemia with jaundice, hematuria, and hemoglobinuria. This plant is being widely cultivated in this country by people of Italian extraction. Snakeroot poisoning is characterized by a slower onset of symptoms and a history of drinking milk from cows pastured in wooded areas where snakeroot is abundant. The symptoms are weakness, prostration, pernicious vomiting, and severe constipation. Mushroom poisoning appears in 6–15 hours after eating poisonous varieties of this plant. It is characterized by hypoglycemia and convulsions, severe abdominal pain, intense thirst, nausea, retching, and vomiting, with profuse watery evacuations. Ergotism is rare in this country and is associated with eating meal or bread made from rye contaminated with the fungus, *Claviceps purpurea.* Water-hemlock poisoning is rare and usually occurs when the leaves or roots of this plant are eaten by mistake. In certain parts of the world honey has been implicated in food poisoning and has been associated with specific flowers from which the bees gather nectar. During World War I rhubarb leaves were for a time recommended as a substitute for greens, and many cases of oxalate poisoning resulted. It is obvious that the symptoms derived from eating poisonous plants or animals may be widely different. An investigator should be familiar with them and with the types of food with which they are associated.

Food poisoning by bacteria or their products.—Bacteria or their products are responsible for the majority of the outbreaks of food poisoning. Certain of these microörganisms are so common that foods cannot be protected from them. For example, staphylococci are in the throat, on the skin, and in the air and thus have ready access to food.

In bacterial food poisoning the foods involved are usually those in which the causative microörganism can grow. Foods that are thought to have caused illness are usually thrown into the garbage pail before investigations are begun. More often than not, a bacteriological examination has to be made from such specimens. Obviously, such specimens are subject to bacterial changes, and the interpretation of laboratory data from such material is, of necessity, open to question. Frequently, a particular kind or brand of food is suspected; it is then desirable for an investigator to obtain a sample from the same source and, if possible, from the same lot for laboratory examination. In some instances it is possible to isolate from such a sample the organism responsible for the food-poisoning outbreak.

People afflicted with food poisoning often erroneously attribute their illness to meat or some meat product which they have eaten. The concept that spoiled meat is a common cause of food poisoning is entirely unfounded and should be discarded. Canned foods are frequently singled out as a cause of illness without any just reason. However, meat and canned foods should not be exempted from consideration and examination, along with other food items, in any investigation of an outbreak.

Botulism and staphylococcus food poisoning result from eating food in which these organisms have grown and produced their toxins. The symptoms are due entirely to pre-formed toxins and not to living bacteria.

Botulism gives rise to characteristic symptoms, such as difficulty in swallowing, double vision, difficulty in speech, labored breathing, and, finally, death from paralysis of the muscles of respiration. These symptoms usually appear within 1–2 days after eating the food containing the toxin. Botulism is caused by eating improperly processed food in which *Cl. botulinum* has grown. Since home-canned vegetables are usually involved, it is important to inquire into the method of canning.

Staphylococcus food poisoning is characterized by symptoms of nausea, vomiting, diarrhea, and acute prostration coming on usually within 2½–3 hours after eating. Staphylococci may grow rapidly in some foods and elaborate enterotoxin within a few hours (5–12 hours or longer) when suitable temperatures are provided. Such foods as milk, cured meats, cream-filled bakery goods, etc., are commonly involved.

Salmonella infection of the food-poisoning type is caused by the ingestion of large numbers of salmonella microörganisms. The symptoms are abdominal pain, diarrhea, chills, fever, frequent vomiting, and prostration. They come on within 7–72 hours after swallowing the living organisms.

Streptococcus food poisoning is less severe than the other types. The symptoms are nausea, sometimes vomiting, colic-like pains, and diarrhea. They come on within 5–12 hours after ingestion of hundreds of millions of these bacteria.

Other types of bacteria have been alleged to cause food poisoning, such as *Bacterium coli* and *Proteus*. The evidence that these microörganisms are causative agents is inconclusive.

OTHER DISEASES TO BE DIFFERENTIATED FROM FOOD POISONING

Epidemics of certain infectious diseases may have some of the properties associated with outbreaks of food poisoning. For example, there are epidemics of acute digestive upsets which are not associated with food or water and are apparently spread by contact infection. These outbreaks are not explosive in nature, and new cases arise over a period of several days.

Bacillary dysentery may occur in large outbreaks, but usually secondary cases follow over a period of days or weeks. An outbreak of bacillary dysentery, therefore, lacks the explosive nature of an outbreak of food poisoning and usually is not related to a specific meal.

Amebic dysentery has a variable period between the swallowing of the cysts of the organism and the onset of symptoms. This period, which may be one of days, months, or years, readily distinguishes it from food poisoning

The early stages of trichinosis may be confused with food poisoning, since a sudden onset of symptoms may follow a specific meal. The onset is usually about 48 hours after ingestion of the larvae. A history of eating improperly cooked fresh pork is helpful in making an early diagnosis. The adult worms may also be sought in the stools in the early stages.

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CHAPTER II

Chemical Poisoning in Food

THE literature on chemical poisoning is extensive. In this book only the common types of chemical poisons associated with foods or those which give rise to symptoms confusing them with microbial food poisoning are considered. The reader is referred to standard textbooks of toxicology for a more complete coverage of this subject.

Chemical poisoning is rare as compared to microbial food poisoning. Some chemicals, such as heavy metals, are occasionally taken with suicidal intent or may be maliciously added to foods. An example of the latter instance is cited by W. D. McNally: A cook who was never apprehended placed arsenic in soup which was served to 100 people at a banquet given in honor of Archbishop M. in Chicago in 1914. In one quart of the soup 1.227 gm. of arsenic trioxide was found (1).

Chemicals have often been mistaken for articles of food or medicine; for example, sodium fluoride (1) has been mistaken for medicine, baking powder, and starch. As reported by McNally, death occurred in as short a time as 45 minutes in one case and as long as 12 hours in another.

In the literature some food-poisoning outbreaks are mistakenly attributed to chemical poisoning, whereas they probably represent microbial food poisoning. This is illustrated in a letter of Dr. J. C. Geiger (2) to the editor of the *Journal of the American Medical Association* relative to a paper reporting cyanide poisoning from hotel silver polish (3). Geiger points out that final proof of food poisoning should not be accepted without positive laboratory evidence.

A critical review of the literature supports the view that many outbreaks of actual food poisoning have been ascribed to chemical agents in the absence of studies adequate to rule out other causative agents. Thus nickel, aluminum, and copper have popularly been linked with food illnesses—a belief which is not upheld by scientific evidence. Many such outbreaks could be better explained on the basis of bacterial food poisoning, as will be pointed out in a later chapter. On the other hand, several chemicals do actually give rise to food-poisoning symptoms. A list of some of these may be found in Table 1.

Antimony poisoning, which, as mentioned in the Introduction, has resulted from cooking in a poor quality of gray-enameled cooking utensils, is characterized by vomiting within a few minutes to an hour after ingesting the chemical. The manufacture of the incriminated product is usually effectively changed when attention is called to such poisoning; but, since unsafe utensils appear on the market from time to time, the possibility of antimony poisoning must be considered.

Arsenic and lead poisoning have been of particular concern among fruit-growers and consumers of sprayed fruit. A group of workers from the Division of Industrial Hygiene at the National Institute of Health have studied this problem over a period of three years. These workers call attention to the fact that in the fruit-growing district around Wenatchee, Washington, where lead arsenate has been in use for more than a quarter of a century, as much as seven million pounds of this chemical have been applied in one year's time, with results which tend to minimize the danger from spraying fruit with these substances. The people of this area, for the most part, eat raw apples which have not been washed. The following is quoted from two paragraphs in the summary of these investigations.

> In an effort to investigate even minimal effects of lead arsenate exposure, the population, particularly consumers, was classified by the number of apples they reported eating annually. Separate systems of classification were set up for persons who reported eating commercially-washed apples almost exclusively; for persons who ate unwashed apples almost exclusively; and for persons who ate both washed and unwashed apples. Chemical analyses of unwashed apples of the kind eaten by residents of the district showed that the spray residue lead averaged about 20 times the amount found on apples shipped in interstate commerce.

TABLE 1

CHARACTERISTICS OF GASTROINTESTINAL UPSETS FROM CHEMICAL POISONS

Specific Agent	Food Concerned	Symptoms	Onset of Symptoms (Hours after Eating)
Antimony	Foods cooked in cheap, gray-enameled cooking utensils	Vomiting	A few minutes to an hour
Arsenic	....	Vomiting, diarrhea, painful tenesmus	10 minutes and longer
Barium carbonate	Pastry of tarts	Vomiting, diarrhea, abdominal cramps with tingling sensations in face and neck, followed by loss of tendon reflexes, disordered action of heart and muscle paralysis	1–2 hours
Cadmium	Acid liquids prepared in cadmium-plated metal utensils	Nausea, vomiting, cramps, and diarrhea	15–30 minutes
Fluoride and sodium fluoride	Sodium fluoride mistaken for baking powder, soda, or flour	Acute poisoning, vomiting, often hemorrhagic, diffuse abdominal pains, and diarrhea occur with great constancy; convulsions, toxic or clonic spasms of certain muscle groups; paresis of certain groups of muscles (eye muscles, facial muscles, hand extensors, and those of lower extremities); hiccough, contraction of pupils, and paresthesia in extremities occasionally present; may be confused with botulism	A few minutes to 2 hours
Lead	....	Abdominal pain, vomiting, and diarrhea in acute poisoning	30 minutes and longer
Methyl chloride	None; leaking mechanical refrigerators	Progressive drowsiness, mental confusion, stupor, weakness, nausea, pain in abdomen, and vomiting; convulsions and cyanosis alternating with coma in severe cases; sometimes confused with botulism	Variable
Mercury	....	Astringent metallic taste, salivation, great thirst, vomiting, and abdominal pain. Watery diarrhea within 2 hours	2–30 minutes after ingesting bichloride of mercury
Nitrates	Water	Cyanosis, vomiting, and diarrhea in infants	Several days
Zinc	Apples cooked in galvanized-iron kettle	Pain in mouth, throat, and abdomen, followed by diarrhea	....

Clinical and laboratory data for 95 men and 145 women classified as consumers were analyzed in such a way as to trace the effects of the increment in lead and arsenic intake superadded to the usual dietary and atmospheric sources of lead and arsenic. The additional amounts of these elements ingested as lead arsenate on sprayed apples and pears raised the concentration of lead in the blood of male and female consumers to an extent which is more conveniently expressed in micrograms than in milligrams, and also resulted in raising the concentration of lead and arsenic in the urine. For statistical reasons (asymmetrical distribution of urinary lead and urinary arsenic values) the latter two findings cannot be studied as closely as blood lead concentration. These additional amounts of lead arsenate ingested as spray residue were not accompanied by any effects on blood findings (hemoglobin content, erythrocyte count, or reticulocyte percentage) or on the occurrence of any clinical findings, whether considered separately or in associations [4].

Arsenic poisoning in man occurs most commonly from causes other than adulteration of food, for example, in metallurgical occupations and in patients under treatment for syphilis with arsenical drugs. Lead poisoning is more often an industrial disease than a food poisoning. The extensive use of lead in paints, batteries, gasoline, glazes for pottery, and insecticide sprays makes these occupations hazardous. Both arsenic and lead when swallowed in appreciable quantities give rise to acute symptoms of nausea, vomiting, and abdominal pain, which may come on within as short a period as 10 minutes.

Barium carbonate poisoning involved a total of 85 British soldiers in two outbreaks (5). In the first, 13 men were admitted to a military hospital with acute gastroenteritis. The men had become ill 1 hour after their evening meal, which consisted of meat, vegetables, and a marmalade tart. Three sergeants who had not eaten the tart were not affected. Five soldiers who had eaten some of the tart but no other part of the meal also became ill. Before laboratory findings were available from the first outbreak, 71 men in the same unit were taken ill 2 hours after a meal. The meal consisted of meat, vegetables, and treacle tart. In this second outbreak only the men who ate the tart were made ill. A chemical analysis of the pastry of the tarts revealed large quantities of barium carbonate. At the supply depot a sack containing 4 pounds of barium carbonate for use as rat poison had been placed, in error, in the flour store, filled with ordinary flour, and issued to the unit.

Cadmium poisoning follows the accidental contamination of acid foods and drinks with soluble cadmium compounds. The history of the preparation of the food or drink and the clinical picture are so similar in all outbreaks that the diagnosis is evident to anyone familiar with this type of poisoning (1). The Division of Industrial Hygiene (6) has listed 346 cases of cadmium poisoning reported in the literature from 1858 to 1941; and, of this number, 287 were due to ingestion, the others being due to either inhalation or ingestion. However, since this compilation, in a single outbreak (7) in a worker's canteen, 300 cases have been reported. This outbreak was caused from wine stored in cadmium-lined containers, the cadmium content ranging from 100 to 180 mg. per liter. S. Frant and I. Kleeman (8) describe four outbreaks occurring in New York City from 1937 to 1940. Lemonade, homemade punch, raspberry-gelatin dessert, and tea containing lemon juice were the items of food contaminated with cadmium. The first three of these foods were placed in ice trays of electric refrigerators for cooling. The ice trays were found to be plated with cadmium. The last item of food was placed in a metal pitcher, the inside of which was plated with cadmium. The amount of this element dissolved by these acid foods was, in order, 300, 67, 530, and 160 parts per million. In the case of the smallest amount (67 parts per million), symptoms came on within 30 minutes, whereas with the larger amount of dissolved cadmium symptoms appeared within 10–15 minutes. The symptoms varied from nausea, abdominal cramps, and weakness in persons consuming small amounts to vomiting, cramps, and diarrhea in patients who consumed the largest amounts. Thirteen to 15 parts per million of cadmium in "popsicles" (a fruit beverage frozen in a small mold around a wooden stick, which serves as a handle) caused violent nausea in 29 school children, with complete recovery in 5–6 hours. In an Air Forces squadron (9) stationed in New Guinea a mixture of fresh lemons, sugar, and water was prepared only 1 hour before serving to approximately 200 enlisted men. Although the symptoms ranged from mild diarrhea to nausea, vomiting, retching, and abdominal cramps, 27 of the men had to be hospitalized, 2 of whom were in complete shock. The essential features are summarized by Frant and Kleeman as follows: "The association of immediate food poisoning of groups with the ingestion of an acid liquid prepared in a metal container should cause suspicion, and an immediate investigation for the presence of cadmium-plated utensils should be made."

Fluoride poisoning (10) is due to sodium fluoride, which is often used as an insecticide. It is a white powder, closely resembling baking soda, for which it has been mistaken. Since sodium fluoride is often used to exterminate cockroaches, the package containing this chemical is frequently kept in the kitchen near the food supplies.

H. B. Baldwin (11) describes an outbreak arising from sodium fluoride which was mistakenly substituted for baking powder in making pancakes eaten by 6 or 7 persons. All vomited within 5–15 minutes afterward. In some cases purging occurred, and in 1 case purging and vomiting continued for a day or more, and the patient, furthermore, complained of pains in the limbs. One individual who ate 3 or 4 pancakes died within 12 hours. Upon investigation, a box of sodium fluoride roach powder was found by the side of the baking-powder box. In another outbreak described by Baldwin (11), a fairly accurate record of the dosage was obtained. In this outbreak, 26 gm. of fluoride were used instead of baking powder in making 26 wheatcakes for breakfast. If the batter had been equally mixed, each wheatcake should have contained 1 gm. of sodium fluoride. The mother of the family ate 5 cakes, one son 9, another son 1, a daughter 6, and the father 9. The father was seized with cramps almost immediately after eating. Purging commenced early and vomiting within 4 hours. The daughter who ate 6 cakes felt sick when eating the last cake and vomited within 5 or 10 minutes. The mother, who ate 5 cakes, was the most seriously affected. She did not vomit for 5 hours, although diarrhea began within 15 or 20 minutes and continued for several days. These outbreaks illustrate the fact that sodium fluoride may be eaten with food and not discovered by taste. Vomiting and diarrhea are often effective in eliminating much of the poison and make it difficult to determine the fatal dosage for man. Baldwin carried out experiments on himself and found that merely tasting small quantities of sodium fluoride produced a slight feeling of nausea, with salivation. As little as 0.25 gm. taken on an empty stomach produced nausea, which was initiated within 2 minutes and reached its greatest intensity in 20 minutes. Vomiting occurred within 2 hours, and nausea continued throughout the following day but disappeared on the second day.

J. C. Geiger (12) reported an outbreak of sodium fluoride poisoning in San Francisco. Poisoning occurred in 21 instances, 3 of which terminated in death. This outbreak was traced to the use of a mixture of sodium bicarbonate and sodium fluoride, sold in bulk as sodium bicarbonate, or baking soda.

A large outbreak (13) of sodium fluoride poisoning involving 263 cases with 47 deaths occurred at the Oregon State Hospital at Salem, Oregon. In this outbreak a patient-helper unwittingly mistook roach powder for powdered milk and added approximately 17 pounds of the compound to a 10-gallon mixture of scrambled eggs. The eggs were rejected by many patients because of a salty or soapy taste, while others complained of numbness of the mouth. Nausea, vomiting, and diarrhea occurred abruptly and at times simultaneously. In many instances blood was present in the vomitus and stools. Soon after eating, the patients complained of abdominal burning and cramps. General collapse occurred in severe cases at varying periods of time and was characterized by pallor, weakness, absent or thready pulse, shallow unlabored respiration, weak heart tones, wet, cold skin, cyanosis, and equally dilated pupils. Death usually followed. When death was delayed and in some cases who recovered, there were paralysis of the muscles of deglutition, carpopedal spasm, and spasm of the extremities. Convulsions, abdominal tenderness, and rigidity were absent. In a majority of fatal cases death occurred 2–4 hours after ingestion of the food, although in a few instances death was delayed for 18–20 hours. In some of the cases local or generalized urticaria occurred, whereas in others there was a thick, mucoid discharge from the mouth and nose.

The cooked eggs contained from 3.2 to 13 per cent sodium fluoride, showing a spotted distribution of the poison. In one patient who died 15 minutes after ingestion of the eggs, 17.2 gm. of sodium fluoride were present in the stomach. In a patient who died 1 hour after ingestion of the eggs, 3.7 gm. of sodium fluoride were found in the stomach. The stomach of a patient who died 4 hours after eating the eggs was empty, but the liver contained 0.85 gm. of sodium fluoride and both kidneys an estimated 0.21 gm. of the poison. In one case in which death was delayed 18 hours, only 0.18 gm. of sodium fluoride was found in the stomach. In all cases fluorides were identified by the etch test on glass from the ash in 10-gm. samples of kidney and liver and by the presence of calcium fluoride bands on spectrographic analysis.

At necropsy in sudden deaths, the mucosa of the stomach, duodenum, and first portion of the jejunum was edematous and hyperemic. When death was delayed, petechial hemorrhages of the gastric and duodenal mucosa were observed. The colon was empty except for portions of undigested food, which could be accounted for by the severe diarrhea. There was a general increased wetness and acute congestion of the abdominal viscera, and the liver and kidneys were swollen. The edges of the lungs were ballooned, and occasional interlobar petechial hemorrhages were present. Slight edema and hyperemia were present in the brain.

Three grams of sodium fluoride are a sufficient quantity to cause death when eaten by man (14). Fluoride toxicity may result from the formation of an insoluble calcium salt and interference with the action of enzymes and lactic acid metabolism. In 34 fatal cases of poisoning due to fluorine compounds (15), vomiting, diffuse abdominal pains, and diarrhea were common. Convulsions and paresis, often alternating, were a characteristic, but by no means constant, finding. Spasms were confined to certain groups of muscles, especially those of the extremities. Vomiting and diarrhea are often so effective in eliminating fluoride that it may not be recovered by chemical tests of the stomach and contents. Sometimes, however, positive tests are obtained.

Pathologically (14), in sodium fluoride poisoning there is congestion and hemorrhagic infiltration of all the organs, particularly the spleen and lungs. Throughout the pulmonary parenchyma the vessels are so dilated and the vascular channels so congested that blood oozes from the alveoli when the tissue is cut. The liver is yellow, but the edges are sharp and the color uniform. There are no areas of necrosis or acute damage, but cloudy swelling is present. The spleen is enlarged and engorged with erythrocytes. The lymphoid elements are not prominent. The pancreas is macroscopically normal. The kidneys are edematous and moderately swollen. The gastric mucosa is congested and hyperemic, as is also the rest of the bowel to a lesser degree. There are small points of hemorrhage in the gastric mucosa, and the stomach contains blood-stained fluid. Symptoms of acute poisoning may come on in a few minutes after the ingestion of sodium fluoride, and in fatal cases death occurs within 6–12 hours or longer.

Sodium fluoride has received considerable study as an anthelmintic for swine (16). It is under consideration as a medicament for the removal of large roundworms, *Ascaris lumbricoides*, from swine when given as 1 per cent of the dry ration for 1 day. This dosage, however, is not without toxic effects on swine, and some animals exhibit symptoms similar to those described for man.

Methyl chloride poisoning is included in this discussion, since the symptoms produced by this type of poisoning have frequently been confused with botulism. Two outbreaks have come to the attention of the author in which a clinical diagnosis of botulism had been made by the attending physicians. However, the symptoms in this type of poisoning are sufficiently different to be distinguishable from those of botulism. In methyl chloride poisoning, nausea and vomiting usually initiate the onset of symptoms, whereas in botulism they may or may not be present. The pupils are dilated, and mental confusion and coma occur in methyl chloride poisoning but not in botulism. In Chicago, 29 cases with 10 deaths occurred in 1929 (17). All these were due to leaks in kitchenette apartment refrigerators in multiple-unit systems using methyl chloride as the refrigerant. Such systems use 100–200 pounds of gas, and a leak in the piping in any one unit may allow great quantities of methyl chloride to escape. Such gaseous poisonings are easily confused with food poisoning if superficially investigated, since many persons in a household may become ill within a short time.

The toxic dosage of methyl chloride for man is not known. W. W. Smith and W. F. von Oettingen (18) present mortality data for dogs and monkeys exposed to methyl chloride in concentrations ranging from 4,000 to 300 parts per million on a schedule of 6 hours daily per week. If man is as susceptible to lethal effects of methyl chloride as dogs and monkeys are, then daily exposure to a concentration of gas of 500 parts per million is extremely dangerous even for a period of 2 weeks or less.

In acute mercury poisoning some of the symptoms resemble food poisoning, especially in the industrial disease, where the pure metal is involved. Similar symptoms also occur when mercuric chloride is taken by mistake for medicinal purposes or with suicidal intent. It is not of importance in food poisoning. The symptoms are listed in Table 1.

Flour bleached with gaseous nitrogen trichloride (Agene) and fed to dogs was found to cause convulsions in them (19). Dogs eating a nutritionally adequate diet containing 75 per cent of "agenized" white wheat flour (with liver powder and casein and fortified with accessory food substances) developed clinical epilepsy within a week, including typical electroencephalographic patterns, indistinguishable from human idiopathic epilepsy (20). Monkeys showed asynergy, weakness, and tremors, sometimes accompanied by cerebral dysrhythmia. Cats exhibit a marked ataxia, and rats an abnormality of porphyrin metabolism. Mixtures of synthetic amino acids and the individual amino acids, cysteine and cystine, after treatment with Agene, when injected intravenously into dogs on such a diet, produces seizures.

Another group of workers (21) produced running fits in dogs fed high levels of flours bleached with nitrogen trichloride and benzoyl peroxide. Fits did not occur in dogs fed untreated flours or flours treated with benzoyl peroxide, chlorine dioxide, chlorine, nitrogen dioxide, or methyl dichloramine. The toxic factor is associated with the protein of wheat but not with the lipid or carbohydrate. Convulsive seizures have been produced in cats fed agenized flour. This group of workers found that monkeys fed agenized flour showed some changes in the electroencephalographic pattens but none of the clinical symptoms associated with running fits. No adverse effect was found by this group of workers on rats, chicks, or guinea pigs. Growth responses of rats and chicks fed nitrogen trichloride–treated whole-wheat flour and unbleached whole-wheat flour were the same. Preliminary studies on 12 human subjects fed agenized wheat products for 2–4 weeks failed to reveal any changes in electroencephalographic patterns, nor were any abnormal clinical symptoms noted.

The Food and Nutrition Board of the National Research Council (22) has taken the position that, in view of the susceptibility of several mammalian species, there is a definite risk of injury to human beings. They have encouraged further study of the problem and urged that safe methods be developed for treating flour.

Nitrates in polluted well water, when present in amounts varying from 0.388 gm. of nitrate ion per liter and higher, have been found to cause cyanosis, vomiting, and diarrhea in infants. Severe symptoms occurred in one infant receiving water with a nitrate nitrogen value of 140 parts per million, which is equivalent to 0.619 gm. of nitrate ion per liter.

Tin poisoning is practically unknown, in spite of the fact that millions of pounds of food packed in tin cans are consumed yearly in this country. Most of the reported outbreaks of food poisoning traced to tin date back many years; and, in view of our present knowledge, these outbreaks were probably due to other causes.

The Bureau of Chemistry and Soils of the United States Department of Agriculture carried out feeding experiments in animals and on human volunteers with food packed in tin containers. The results, which were negative, apparently have not been published. In a letter concerning these tests three experiments were detailed, one of which is as follows:

> In order to determine the urinary and fecal elimination of tin a human diet squad of four subjects was used. In this experiment the four subjects were placed on a diet shown analytically to be tin free for a preliminary period of 9 days. This was followed by a tin-feeding period of 6 days in which canned pumpkin and canned asparagus were fed. The canned pumpkin was in two separate lots, one of which contained 476 parts per million and the other 383. The canned asparagus contained 361 parts per million of tin. During this tin-feeding period the four subjects ingested a total of 2278 to 2942 milligrams of tin with an average daily ingestion ranging in the four subjects from 426 milligrams to 490 milligrams of tin. During this tin-feeding period the diet was supplemented by such of the tin-free food as the subjects desired. Following

the tin period there was a return to the tin-free diet for 10 days which period was divided into three intervals, that is, ending the third, sixth, and tenth days. The urine and feces eliminated by each of the subjects were composited for each of the periods indicated and analyses made for tin. In the urinary samples no tin was found in either the pre-tin, tin, or post-tin periods. In the tin period fecal analyses showed the greatest elimination of tin with diminished amounts in the three samplings of the post-tin period with measurable quantities of tin still present ten days after the tin-feeding period. The elimination of tin through the feces in these human experiments showed 76, 73.8, 91.4, and 91.2 percentages, respectively, for the four subjects. It may be stated that neither during the tin-feeding period nor during the post-tin period did any of the subjects show any evidence of illness or discomfort even after ingesting such abnormally large amounts of tin-containing food [23].

In the next paragraph of the letter the following statement is made:

> Our own experimental work, involving the ingestion of far larger amounts of tin than any previously reported, and supported by the experimental evidence of other investigators, leads us to the conclusion that tin, in the amounts ordinarily found in canned foods and in the quantity which would be ingested in the ordinary individual diet, is for all practical purposes eliminated and is not productive of harmful effects to the consumer of canned foods [24].

There is a common concept among the laity that food spoils more quickly when standing in an open tin can than if removed from the can and stored in a separate dish. This is a myth of long standing. Spoilage results from bacteria growing in a food, and, once the food is contaminated, time and suitable temperature will cause spoilage, regardless of whether the food is in a tin can or in a glass or porcelain dish. In fact, contamination is less likely to occur if the food is left in the sterile can than if placed in a clean but not sterile dish.

Zinc poisoning in food is so rare that it is usually reported when it is detected. It is associated with acid foods cooked in galvanized-iron kettles. In one outbreak (25) 200 people became ill a few minutes after eating applesauce which was found to contain 1.62 gm. of hydrated zinc sulphate per pound of apples. In another outbreak (26), more or less severe symptoms of poisoning developed in 25 of 42 men who had eaten cooked apples. Chemical tests revealed 83 mg. of zinc in 100 gm. of the food and 17 mg. of zinc in 100 gm. of vomitus. In both outbreaks galvanized-iron kettles had been used for cooking the apples.

Miscellaneous chemicals were in more or less common use many years ago for the preservation of foods (27). Among these were borax or boric acid in meats, milk, butter, oysters, clams, fish, sausage, and other foods; formaldehyde in milk; salicylic acid in jams, fruit juices, soda-water syrups, cider, wines, and other sweet preparations; potassium permanganate on the surface of meat to destroy evidences of decomposition; sulphurous acid to preserve and retain the color of fresh meats; and hydrogen peroxide to preserve milk, wine, beer, and fruit juices. Since the passage of the Food and Drug Act of June 30, 1906, the use of these has been discontinued.

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CHAPTER III

Poisonous Plants and Animals

SOME plants, such as snakeroot, poisonous mushrooms, water hemlock, and the leaves of rhubarb, give rise to gastrointestinal symptoms. Other plants and animals, such as shellfish, fava beans, and a parasitic fungus of rye, elicit various types of poisoning but no gastrointestinal upsets. Data relevant to the poisoning associated with the more important of these forms may be found in Table 2. Other plant or animal poisonings which are rare or not well established are omitted from this discussion.

SHELLFISH POISONING

In July, 1927, in San Francisco and vicinity a sudden appearance of approximately 102 cases of mussel poisoning occurred, which attracted much attention in the daily press and gave rise to an investigation by the California State Department of Health and the State Fish and Game Commission. Meyer and others (1) have tabulated 20 outbreaks of mussel poisoning in the world, beginning with one reported as far back as 1793. Since 1793, approximately 244 people have been poisoned, and 38 have died. Of the 102 people in the 1927 California outbreak, 56 were men (aged twenty-four to sixty-five years), 8 boys (aged two to twelve years), 34 women (aged twenty to sixty years), and 4 girls (aged thirteen to fifteen years). All those taken ill had eaten steamed, cooked, or raw mussels of a Pacific Coast variety (*Mytilus californianus*), freshly gathered in different localities approximately 45 miles south and 50 miles north of the Golden Gate.

From 1927 to 1947 the records for the Pacific Coast (2) show

TABLE 2

CHARACTERISTIC SYMPTOMS DUE TO POISONOUS PLANTS OR ANIMALS

Disease	Specific Agent	Food Concerned	Symptoms	Onset of Symptoms (Hours after Eating)
Shellfish poisoning	Plankton (*Gonyaulax*), food of mussels	Mussels	Respiratory paralysis; milder symptoms consist of trembling about lips to a complete loss of power in the muscles of extremities and neck	5–30 minutes and longer
Favism	Bean, *Vicia fava*, or inhalation of pollen from blossoming plant	The bean *Vicia fava*	Acute febrile anemia with jaundice, hematuria, and hemoglobinuria	1 hour after eating beans in one case
Snakeroot poisoning	Trematol from snakeroot (*Eupatorium urticaefolium*)	Milk from cows pastured on snakeroot	Weakness or prostration, pernicious vomiting, severe constipation, and epigastric pain; temperature normal	Variable period; after repeated use of milk from cows pastured in area where snakeroot is present
Mushroom poisoning	Phalloidine and other alkaloids	Poisonous mushrooms (*Amanita phalloides* and other species)	Hypoglycemia and convulsions; severe abdominal pain, intense thirst, nausea, retching, vomiting, and profuse watery evacuations sometimes containing blood and mucus	6–15 hours after ingestion
Ergotism	Parasitic fungus of rye (*Claviceps purpurea*)	Rye meal or bread	Gangrenous—involves limbs, especially fingers and toes, occasionally ears and nose. Convulsive—depression, weakness and drowsiness, headache, giddiness, painful cramps in limbs, and itching of skin	Gradual, usually after several meals of diseased rye in meal or bread
Water-hemlock poisoning	Cicutoxin or resin from water hemlock (*Cicuta maculata*)	Leaves and root of water hemlock	Nausea, vomiting, and convulsions	1–2 hours

409 cases of mussel and clam poisonings with 35 deaths. Most cases occurred along the central California Coast, with a sprinkling of severe outbreaks from Juneau, Alaska, to Southern California and the Gulf of California, Mexico. All cases appeared between May 15 and October 26.

Shellfish poisoning has been reported in certain shellfish in Nova Scotia (3). In July, 1936, Dr. P. S. Campbell, chief health officer of Nova Scotia, reported to the Department of Pensions and National Health the occurrence of a few cases of severe illness in Digby County following the consumption of mussels. Two of the cases were fatal. The poison was found to be similar to that reported in California. Two species of mussels were found to be toxic, i.e., *M. edulis* and *Modiola modiolus*.

In a survey (4) of commercially important shellfish areas of this region, paralytic shellfish poison has been found in shellfish only from the Bay of Fundy area in the late summer and early fall. In August, 1945, in New Brunswick, 21 cases of poisoning were recorded. All these cases resulted from consumption of clams whose toxicities were above 6,000 mouse units (MU).* The mean consumption of poison required to produce mild, severe, and extreme symptoms of poisoning in susceptible persons was found to be about 7,000, 16,000, and 32,000 MU, respectively.

A history of a typical case, taken from Meyer and others (1) (case 2), is as follows:

> A middle-aged well-built man called at the laboratory. He had an ataxic gait but spoke distinctly and stated that he had been poisoned by eating mussels. On Sunday, July 17, at 1:00 P.M. during low tide he gathered mussels which were submerged at a place approximately three miles west of Pescadero. No sewage discharges into the sea within twenty miles. Late in the afternoon the carefully washed shell-fish were steamed for about 12 or 15 minutes. The hot and thoroughly cooked mollusks tasted good and he ate about 2 quarts (three dozen small size) and his wife about 6 small mussels during the evening meal. About 15 minutes after eating he felt nauseated but could not vomit. He felt drowsy, went to bed, and had a restless sleep continually interrupted by dreams. Although he did not feel well in the morning he went to his busi-

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* The mouse unit (MU), or average lethal dose, has been defined (H. Sommer and K. F. Meyer, Arch. Path., 24:560-98, 1937) as that amount of mussel poison which when dissolved in 1.0 ml. of water and injected intraperitoneally into a 20-gm. white mouse will cause death in 15 minutes.

rose. About 10:30 a.m. he noticed a general numbness around his lips, chin and cheeks and particularly in the thumb of the left hand. These symptoms still persisted during his examination on July 18. The reflexes were present and active, the pupils reacted to light and were dilated. The temperature was 98.4° F. The numbness gradually disappeared during the afternoon. The broth (0.5 cc.) of the steamed mussels killed a mouse within 3 minutes.*

During the meal the patient fed several mussels to 3 half grown kittens and one cat, and two mollusks to a police dog. The dog vomited within 20 minutes but was not otherwise affected. The 3 kittens died during the night at the same place where the food had been offered in the evening. The cat showed a complete posterior paralysis 18 hours after eating mussels, but recovered.

During the years which have followed the initial outbreak, work on this problem has been continued at the George Williams Hooper Foundation at the University of California. Many theories have been advanced to explain the occurrence of the poison in shellfish. The hypothesis that the toxic agent is an organism pathogenic for mussels or the assumption that the toxicity is due to an actual disease have not been supported by fact. Many theories were abandoned as it became more apparent that the agent responsible for the toxicity is contained in the ocean water and approaches the shellfish beds more or less periodically from offshore. The search for the causative factor narrowed down to a dissolved or particulate substance in the water which the mussels were absorbing. The food of the shellfish, i.e., the diatoms and dinoflagellates, were next considered. The poison has been detected in and extracted from plankton (5). Samples of plankton collected frequently during the summer months of 1935 yielded amounts of poison roughly proportional to the numbers of the dinoflagellate, *Gonyaulax catenella*, present. On the average, 3,000 of these organisms yielded 1 MU of poison. Sommer and others believe that a certain species of the genus *Gonyaulax* is the source of the paralytic shellfish poison. They also present evidence of other toxic substances in plankton. The dinoflagellate, *G. catenella* (6, 7), is a free-swimming organism of dark orange or greenish-brown color, which multiplies by the formation of chains of two, four, or even eight individuals and lives like a true plant cell by photosynthesis. It may multiply to such an extent that the water presents a deep rust-red color, so-called "red water," which may be seen for miles in the daytime and is a beautiful, luminescent spectacle at night.

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* The symptoms of numbness around the lips, chin, and cheeks, of which this man complained, have in other cases appeared in less than an hour.

A method has been devised for detecting and quantitatively estimating the poison in shellfish. It consists in measuring amounts of alcohol-soluble extracts of the livers of the shellfish by injecting mice intraperitoneally.

The poison.—This poison (5) is one of the strongest known. It belongs to the class of alkaloids, such as strychnine, muscarine, and aconitine. It is readily soluble in water and alcohol but is insoluble in ether and chloroform. The stability of the poison (8) in aqueous solution decreases with an increase in pH and an increase in temperature. The mussel poison has been concentrated by the use of ion exchange on a synthetic zeolite (barium or sodium Decalso). With this method, it was possible to obtain a 50–70 per cent yield of the poison with a toxicity of 70–140 MU/mg, an increase in potency of eighteen fold. The fatal dose for man is probably a few milligrams by mouth.

A procedure (9) has been developed for the chromatographic fractionation of mussel poison concentrates on active carbon. The adsorption behavior of mussel poison on active carbon was found to vary widely with different anions. By chromatography on Norit A of mussel poison concentrates obtained from ion exchange on Decalso, it was possible to obtain a yield of 30–50 per cent of twenty-fold enriched material with a toxicity greater than 1.00 MU/μgm. The chromatography of partially decolorized, defatted, crude mussel poison extracts on Norit A was found to yield 30–50 per cent of seventy-five-fold enriched material.

Mussel poison has been crystallized (10) as the rufianate, helianthate, and reineckate without losing its pharmacological activity. Mussel poison sulphate has been prepared. Large-scale purification of mussel poison has been accomplished by crystallization of the helianthate.

A large-scale collection of marine plankton (11) rich in *G. catenella* was made by passing water containing large numbers of the plankton through supercentrifuges. Concentration by chromatography on acid-washed Norit A furnished a fraction of poison with a toxicity of 1.64 MU/μgm. Choline and trimethylamine were identified as two of the bases present in marine plankton. The purest preparation of the mussel poison (12) was found to have a toxicity of 4 MU/μgm. The chemical structure of the toxic principle is still to be determined.

Sampling.—Since the variation in toxicity of mussels in a given bed is not large, the standard sample is limited to a few mussels, whose length, weight, and volume are considered. Volume seems to be a better criterion than weight, since it is not affected by shell water as the weight is. It is measured by immersing the mussels in tap water in a graduated cylinder. The shells of live animals under such conditions are always tightly closed, and no error is likely to occur from imbibition of water. Sommer and Meyer use the following procedure for sampling: Generally, three or four mussels are selected, the combined length of which measures 10 in. (25 cm.). The volume is determined and the results of the test expressed in units of poison per 100 cc. of whole mussel.

Test for poison in clams or mussels (13).—The livers of the mussels or the livers plus syphons of the clams are used. Ten ml. of 0.1 *N* hydrochloric acid per shellfish are customarily employed for extraction. The tissue is ground with the dilute acid and the mixture boiled gently for approximately 5 minutes. After centrifugation or filtration, the liquid is ready for injection. The results are expressed in mouse units per 50 gm. of mussel or per clam; the poison content may vary from less than 10 to over 10,000 MU per shellfish.

In the case of canned or fresh clams the following standard test has been widely adopted: One hundred gm. of ground clam meats are extracted with 100 ml. of hydrochloric acid 0.1–0.2 *N*, and the extract is prepared as above. (The strength of the acid depends on the species of clam, 0.1 *N* for Little Neck clams, 0.15–0.2 *N* for Washington clams.) The mixture should at all times react acid to congo paper. The results are expressed in mouse units per 100 gm. of clam meats.

Sommer and Meyer analyzed mussels from numerous places between central California and southern Oregon. Besides the shellfish mentioned, seven of the common varieties of edible clams were found to contain the poison in smaller amounts.

Poisonous mussels were found to be indistinguishable from normal ones, except by the animal test. Mussels subjected to various conditions in the laboratory have never shown an increase in toxicity; they usually show detoxification, the rate of which has been determined. The poison was also demonstrated, during the poison season, in the residue of filtered sea water.

No specific treatment has been found for this disease.

FISH POISONING

Fish have frequently been implicated in food poisoning and particularly in tropical or subtropical areas. No clear-cut experiments have demonstrated specific etiological agents. Robert L. Gilman (14, 15) has reviewed fish poisoning in the Puerto Rico–Virgin Islands area. He quotes Mann's observations of the symptoms as follows:

> *Onset.*—The onset is from 1 to 6 hours after eating the fish. The patient appears critically ill with severe gastro-intestinal symptoms of nausea, vomiting, and diarrhea. There is a distinct metallic taste. The skin becomes flushed and there is tingling and itching that may last for days. Cramps in the extremities may occur. Subsequently there may be weakness in the legs with possible temporary paralyses with reduced or absent knee jerks. Hyperaesthesias and paraesthesias are commonly associated. (The latter causing the patient to interpret a cold object as feeling warm or cold food as being hot.) There may also be associated nervousness, restlessness, or insomnia; albumin and casts and frequency of urination are prominent symptoms.
>
> *Course.*—The course may run from 1 to 3 weeks with gradual recovery. No fatalities have been recorded. Mann regards the metallic taste and the paraesthesias (in which cold feels hot or gives a hot sensation in the mouth) as being the significant pathognomonic symptoms of fish poisoning. It does seem as if this definite chain of symptoms and their character may furnish a valuable lead in identifying the nature of the toxin involved.
>
> The symptoms differ from those reported following ingestion of certain *tetrodon* fish found in Hawaiian and Japanese waters in which the active toxin, a leucomine, has been isolated. In these instances the onset often appears within 15 minutes, and while gastro-intestinal symptoms predominate there is a marked "burning sensation" and the mortality is high and fairly prompt.

Gilman has listed 11 facts or beliefs with reference to the etiology of fish poisoning as follows:

1. The poisoning is not confined to a single species or related species.
2. Not all fish of the same species and size, caught at the same time and place are poisonous.

3. Variations exist in the relative frequency of poison cases such as occasional examples following the eating of kingfish, a higher incidence after eating the large barracudae, and a more frequent occurrence following the eating of a *caranx*.
4. The occurrence of fish poisoning is definitely seasonal and varies with the location and depth of fishing.
5. The smaller-sized fish are much less apt to prove poisonous (never, some believe), while the larger-sized are more apt to prove poisonous, especially those spawning.
6. While not all those eating fish that have been established as poisonous are affected to the same degree, both whites and natives seem equally susceptible.
7. One experience of poisoning confers no immunity.
8. There exists no evidence that the poison is bacterial in origin, nor does thorough cooking prevent the poisoning.
9. It is believed, however, that a shortened interval between the catch and its consumption minimizes the poisonous effects (theory).
10. The symptomatology is definite, distinctive, pathognomonic.
11. The natives believe that certain changes are visible in the flesh of the fish indicative of their poisonous character.

The symptoms and facts or beliefs listed are suggestive of staphylococcus food poisoning.

FAVISM

This disease, occurring mainly among Italians, is attributed to eating the fava bean or smelling the blossoms of the bean plant. A large Mediterranean population lives in the United States, and the fava beans are cultivated extensively in New York, New Jersey, Illinois, and California (16). They are a staple article of diet and are imported as canned food from Italy. In 1,211 cases reported in the United States (17), 38 per cent developed after the inhalation of pollen and 62 per cent after the ingestion of raw or cooked beans. The prevalence varies from year to year, and the attack occurs most frequently after the ingestion of the first green beans of the season. Poisoning is more likely to occur in individuals who consume them infrequently.

When fields of fava beans are in blossom, the inhalation type of the disease is most prevalent. The pollen is said to be sticky and is not widely disseminated. The first symptom appearing after inhalation of substances originating from the flowers or plant of the fava bean is dizziness, sometimes reaching the stage of collapse. This may occur within a few minutes or some hours after exposure and is usually followed by the gradual appearance of headache, malaise, and nausea. Repeated yawning, vomiting, chill, pallor, and pain in the lumbar region are followed by high fever. The most specific symptoms are hemoglobinuria, which occurs within 5–40 hours, followed by icterus.

T. McCrae and J. C. Ullery reported a case with the following history:

> Dec. 12, 1932, J. C., a white man, aged 53, was admitted to the Pennsylvania Hospital in a serious condition. He had a ghastly appearance due to a combination of jaundice and a curious ashen gray color, particularly of the face. He showed extreme weakness and complained of frequency of urination and the passage of black urine. Lesser complaints were of anorexia and constipation. He was intelligent and able to give a clear history. Dec. 7, 1932, he had eaten a very hearty meal, one of the principal constituents of which was cooked beans, of which he had taken a large amount. An hour after the meal he began to feel marked weakness and was compelled to lie down. Two hours later he voided urine and noticed that it was very black. From this time until his admission to the hospital he was compelled to be in bed, the passage of black urine had continued and there had been a marked frequency of urination, usually about 19 times in the 24 hours. December 7 he had taken a large dose of Epsom salts and stated that blood was present in one of the stools after this. Shortly after the onset he began to have pain in the back, which had been constant. His weakness had increased markedly and since the second day of his illness he had not been able to get out of bed. On Dec. 10, jaundice was noted for the first time and this apparently increased rapidly.
>
> The patient volunteered the statement that he had had similar attacks during his childhood in Sicily and associated his present illness with these. At the age of 7, while walking in the country, he passed a field of plants in full bloom. Soon after this he began to feel dizzy and weak, and he was able to walk a short distance only when he lost consciousness. He was found lying on the ground and was carried home, regaining consciousness later in the day. Following this he passed bloody urine for 3 or 4 days and felt marked weakness for about 10 days, after which he recovered his usual health. He stated that he had identical attacks each year from the age of 7 until he was 14, having one attack each year under the same circumstances. On each occasion he lost consciousness but did not know the exact duration of unconsciousness until the last attack, when he was told that it persisted for 15 hours. He came to the United States at the age of 14 and had had no similar trouble until his present illness. With the onset of this illness he at once recalled the experience of his childhood and associated the beans which he had eaten with the plants which had affected him when in flower. He thought the beans which he had eaten were the same as those which grew on the plants in Sicily [18].

McCrae and Ullery (18) mention the fact that hypersensitiveness and anaphylactic shock can be produced by feeding the beans or injecting the protein of the bean first and later feeding the beans. They also mention that the disease rarely occurs in persons who eat the beans regularly but often follows after the first time the beans are eaten in a given year. The theory that this disease results from an allergy seems to be confirmed by a second case reported in the United States by Hutton (17). A man twenty-one years of age had eaten fava beans and became suddenly ill at a ball game. He complained of weakness, a yellowish tinge of the skin, and abdominal distress. The symptoms were similar to the previous case. The interesting feature of this case was the family history. The maternal grandmother had had several attacks of sudden weakness, accompanied by pallor, jaundice, and dark-brown, bloody urine. The acute picture lasted 3 or 4 days and was followed by rapid recovery. The mother had had similar attacks. The eldest brother had had one attack of the same nature. The maternal great-grandfather and his sister and all her children had had attacks almost yearly in the spring during the fava season. One of the maternal grandmother's children died in an attack after a neighbor had given her a few raw, green fava beans to play with. She ate these beans and died in 24 hours with collapse and jaundice.

Many cases of this disease in the United States are probably not recognized. Although there appears to be a hereditary basis for sensitization to this plant, the symptoms follow a definite pattern peculiar to this specific type of poisoning. Hutton reports that a hereditary factor is present in 20 per cent of the cases. In certain families every member for generations has been reported severely affected. On the other hand, individual susceptibility varies, and certain people, after years of eating the beans with impunity, suffer a single severe attack and none subsequently. Ingestion of the beans by immune mothers may affect nursing infants. There is no sex predilection.

TREMATOL, MILK SICKNESS, OR SNAKEROOT POISONING

This disease is due to trematol ($C_{16}H_{22}O_3$) (19), which is one of the toxic constituents of white snakeroot, *Eupatorium urticae-*

folium, found in the central states of the United States, and of jimmy weed, *Aplopappus heterophyllus*, found in certain sections of western Texas, New Mexico, and Arizona. Cattle apparently will eat these weeds when grazing is scarce. White snakeroot is often abundant in heavily wooded areas. The poison produces "trembles" in cattle and milk sickness in man if he drinks milk or eats milk products from poisoned cows. Pasteurization does not destroy the poisonous properties. The symptoms of the disease in man are weakness or prostration, pernicious vomiting, severe constipation, and epigastric pain. The temperature is characteristically normal or subnormal. Muscular pains are common. There is great thirst, and the breath may have a distinct odor of acetone. The urine is scanty and may contain acetone. The cheeks are flushed, and there may be a marked redness of the lips and tongue. In fatal cases coma and convulsions precede death. In patients who recover, weakness persists for days, weeks, or even months, depending on the initial severity of the illness.

The history of milk sickness is very interesting and is reported in an early paper by E. O. Jordan and N. M. Harris (20). This disease followed emigration in its westward march from North Carolina to Tennessee and Kentucky. It prevailed in various parts of Ohio, Indiana, and Illinois. The mother of Abraham Lincoln died of milk sickness at Pigeon Creek in southern Indiana in 1818. Whole villages in Illinois were depopulated by the ravages of this disease, which was responsible for nearly one-fourth of the deaths of pioneers and early settlers in Madison County, Ohio. In an old port in Dubois County, Indiana, in 1815, more than half the deaths were alleged to be due to milk sickness. The toll of livestock taken by "trembles" was also a great handicap to the pioneers. Trematol poisoning is known in the West as "alkali poisoning." Although the cause of the disease has been ascertained, sporadic cases still occur. G. H. Gowen (21) reported twenty-one cases in Illinois, in which there were two deaths. In every outbreak which he investigated, the animals which were responsible had been turned into a wooded area and were entirely dependent upon what they could forage.

MUSHROOM POISONING

Mushroom poisoning follows the eating of poisonous mushrooms which have been gathered in the woods and fields and mistaken for edible varieties. There are some seventy to eighty species of mushrooms that W. W. Ford (22) has found to be poisonous to man. The southern European population which has migrated to this country often confuses the poisonous ones with the European edible varieties. The differentiation of the various types of mushrooms is a matter for a trained mycologist and not for the layman.

The most poisonous of the mushrooms is *Amanita phalloides*, sometimes called "white Amanita" or "deadly Amanita." In fact, two or three are sufficient to cause serious and fatal illness in the adult. Symptoms often appear within 15 minutes after eating the mushrooms but sometimes are delayed from 1 to 6 hours. This poisoning is characterized by salivation, excessive perspiration, a flow of tears, nausea, retching and vomiting, pain in the abdomen, and violent movement of the intestines, which causes profuse watery evacuations. The pupil is contracted. Respiration is often quickened and dyspneic. Dizziness and confusion of ideas are often noted. Coma may occur and death follow within 48 hours. In fatal cases, fatty infiltration with central necroses is found in the liver, epithelial necrosis of the kidney, acute enteritis, and colitis. The uremic condition resulting from the kidney damage may be responsible for the symptoms. The case fatality in *A. phalloides* poisoning is from 60 to 100 per cent.

Kobert in 1891 (23) isolated a powerful hemolytic substance from *A. phalloides* which he called "phallin." W. W. Ford (24) extracted the hemolytic substance and reported that it was of the nature of a toxin, since it acted upon the animal body after a definite latent period, produced lesions characteristic of bacterial intoxications in general, and induced immunity in susceptible animals by the inoculation of nonlethal doses. J. J. Abel and W. W. Ford (25) concluded that it was not a toxalbumin, as Kobert had supposed, but a nitrogenous glucoside which is very sensitive to the action of heat and acids and less so to the action of alkalies. It is easily decomposed by acids to a pentose and a volatile base or bases, such as ammonia and methylamine. They found it in fresh specimens of *A. phalloides* and *A. citrina* and designated it "Amanita hemolysin." Since Seibert (26) failed to detect its presence in fresh extracts of *A. citrina*, the fungus may differ in its content of glucoside in different parts of the world. H. Schlesinger and W. W. Ford (27) studied the Amanita toxin and found that it could not be a proteid, a glucoside, or an alkaloid. They make the following statement: "Nevertheless, since we are dealing with a conjugate sulfate which, on fusion with dry potassium hydrate, gives off pyrrol and indol we are led to conclude that Amanita-toxin, although not necessarily an indol derivative, is at least an aromatic phenol so combined with an amine group that it readily forms an indol or pyrrol ring."

Muscarine, which is a syrupy, alkaloidal-like substance, has been obtained in a crystallizable form as a hydrochloride from the mushroom, *A. muscaria*. In its action it resembles pilocarpine, which stimulates the myoneural junctions. Treatment consists of stomach lavage, castor oil for eliminating any residual toxin from the gastrointestinal tract, and atropine sulphate (0.0005 gm.) subcutaneously.

ERGOTISM

Ergotism is a rare type of poisoning, at least in this part of the world. It occurs as a result of eating a parasitic fungus, *Claviceps purpurea*, which causes a disease of rye and occasionally of other grains. The fungus grows in the grain as a blackish mass, which discolors the meal. A discussion of this poisoning is given by Barger (28). The essential features of ergot poisoning are listed in Table 2.

WATER-HEMLOCK POISONING

The water hemlock, *Cicuta maculata*, is often erroneously called "parsnip," "wild parsnip," or "wild carrot." Its habitat is in swamps, along irrigating ditches, and in wild meadows. Numerous references to the literature may be found in a paper by L. M. Gompertz, who describes an outbreak involving seventeen boys. His description in part is as follows:

> June 16, 1925, I was called to attend 17 boys, inmates of the New Haven County Home, who had been taken suddenly ill. The boys ranged in age from 9 to 13 years. All of the children in the institution had been dismissed from

> their classrooms at 3:30 P.M., apparently well, and immediately adjourned to their playground. At 5:15 the boys were in the dining room at their evening meal. The matron in charge observed that several of them refused their supper and appeared "pale and sickly." Two of the children asked permission to leave the room, complaining of nausea, but before going very far fell to the floor in violent convulsions. About 20 minutes later, when I arrived, five children were in convulsions; twelve others were vomiting and appeared seriously ill. It was evident that the children were suffering from some form of poisoning, and I soon learned that they had partaken of a plant, the nature of which was unknown to me at the time. It later appeared, however, that in a plot of swamp land adjoining their playground water hemlock grew in abundance. While some of the boys had eaten the roots, the majority had partaken only of the leaves or flowers. It is noteworthy that the five boys who had eaten the root stock were very sick with accompanying convulsions, while the other twelve, who had eaten leaves or blossoms but no roots, did not have convulsions [29].

All 17 boys made complete recoveries.

In Gompertz' (29) review of the literature he states that the poisonous component of *Cicuta* is a resin, which was first isolated and named "cicutoxin" by Boehm in 1876. Cutting the rootstock causes the exudation of an aromatic, yellowish, oil-like substance with an odor similar to parsnip. Cicutoxin is a clear, brown, sticky resin having an acid reaction. It is soluble in ether, alcohol, chloroform, and dilute alkalis.

POISON HONEY

A curious type of poisoning from honey has been described in the United States (8 cases in New Jersey in 1896), in Brazil, Asia, and Africa. It appears to be common in the vicinity of the Black Sea. The poison appears in the honey derived from certain nectar-bearing plants (30), such as varieties of azalea, rhododendron, oleander, and perhaps mountain laurel and palmetto. Honey from the flowers of the black locust has sometimes been toxic.

RHUBARB POISONING

During World War I the use of rhubarb leaves as greens was recommended. In 1917 an editorial (31) appeared in this country and is quoted in part as follows:

> Certain newspapers and magazines in this country are advocating the use of rhubarb leaves for greens. Advice to use the leaves as a substitute for spin-

ach has been promulgated from at least one popular educational center. Because the rhubarb leaves contain certain substances—presumably oxalic acid—in sufficient quantity to make them toxic at times to some persons at least, and because disastrous results have followed their culinary use, the U.S. Department of Agriculture has issued a warning to housewives against using this portion of the plant for food [32]. How timely these instructions are is borne out by the report of a death from eating rhubarb leaves recently reported in the *Journal* [33].

H. F. Robb (34) reports a case of rhubarb poisoning in a woman who ate rhubarb leaves which had been fried and prepared as greens. Within 12 hours she had cramplike pains, which continued intermittently for a period of 36 hours or longer. Death occurred in about 48 hours. A few hours before death the temperature was normal, the respiration 36 a minute, and the heartbeat 120 a minute. A brown bloody fluid was vomited, and a 6-month fetus was aborted. The blood from the placenta failed to clot. After death some blood coming from the nose also failed to clot.

In the same year another report (35) was made of the poisoning of 9 people who ate canned rhubarb. These victims ranged in age from two to eighty-six years, and their symptoms came on uniformly within a 20-minute period, commencing about 2 hours after eating the rhubarb. Following the free use of a stomach pump, lime water, and castor oil, all recovered.

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CHAPTER IV

Botulism

THIS disease is due to toxin formed by the growth of the botulinum microörganisms usually in underprocessed canned foods. By the time that symptoms appear, the toxin has produced damage which often cannot be overcome by specific antitoxin therapy. War conditions in 1917–18 served as a stimulus to preserve more food, and at that time the danger of botulism was not recognized. Hence considerable spoilage from underprocessing was encountered, with an occasional outbreak of botulism. Research to determine the cause of spoilage showed that the cold-pack method of processing foods for home canning, as recommended by the government at that time, was entirely inadequate to prevent botulism. Coincident with outbreaks of botulism from home-canned products in 1918–19, commercially canned ripe olives and spinach were found to be responsible for cases of human botulism. Whereupon, the commercial canning industry, through the National Canners Association, established grants at several universities* for a study of the cause and prevention of botulism. Valuable contributions have also come from the United States Public Health Service. Aided by this research, commercial canners have practically controlled the disease in their field, as is attested by the fact that, with one possible exception (1), no outbreak of botulism has been reported since 1925 from commercially canned goods packed in the United States. Unfortunately, the record from home canning is not so good and constitutes the greatest problem today in botulism.

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* Harvard University, University of Chicago, Stanford University, and University of California.

The United States Department of Agriculture and state health departments have done commendable work in educating housewives to the danger of botulism and the need for safe processing of canned goods. This educational work is necessarily slow, and, unfortunately, on the average, about fifteen outbreaks have occurred each year during the last ten years. The number of annual outbreaks during this ten-year period is tabulated in Table 3 (2).

HISTORY

Botulism has been known in Europe for many years, the first recorded case being in 1735 (3). The term "botulismus" (*botulus*, a "sausage") was coined by physicians of southern Germany in the beginning of the nineteenth century to signify a type of food poisoning which was caused by the ingestion of spoiled sausages. Other names in use in the literature are "allantiasis," "ichthyosismus," and *Wurstvergiftung*. In the early years the term was restricted to sausage poisoning, but the symptom complex produced by sausage poisoning has been found to apply to poisoning involving other preserved foods, such as smoked meat and fish, and to underprocessed canned vegetables and meat.

TABLE 3

Year	No. of Outbreaks	Year	No. of Outbreaks
1937	15	1943	13
1938	16	1944	22
1939	18	1945	13
1940	18	1946	9
1941	15	1947	11
1942	16		

In 1895 Van Ermengem (3) succeeded in isolating a bacterium from ham which had caused the illness of twenty-three persons, in all of whom the symptoms were those of true botulism. He proved that the poisoning in botulism is due to a toxin which is produced when the bacillus is grown upon a suitable culture medium.

A careful survey of the literature on botulism from early times up to 1918 has been made by Dickson (3) and has been reviewed by Meyer (4) for the following ten-year period. In December, 1919, following outbreaks of botulism resulting from the consumption of factory-preserved ripe olives, a commission was formed on the Pacific Coast. The subsequent investigation was sponsored by the National Canners Association, the Canners League of California, and the California Olive Association. The purpose of the investigation was to determine the danger from botulism, how it arises, and how it can best be avoided and overcome. The report of the commission was included in a bulletin (5) entitled *The Epidemiology of Botulism*, published in 1922.

GEOGRAPHICAL DISTRIBUTION

Botulism occurs throughout the civilized world, and the reported outbreaks have been collected by Meyer (Table 4). In Germany most of the outbreaks were from sausage, whereas in Russia (6) they were from fish, such as salt herring, salt salmon, smoke-dried herring, dried sturgeon, and smoked sturgeon. One outbreak was caused by canned onions (7) (shallots), which had been packed in Naples, Italy, and sold in the United States. Several of the cans in this shipment were found to contain potent toxin of *Clostridium botulinum*. K. F. Meyer (2) has collected data on 462 outbreaks occurring in the United States and Canada between 1899 and 1947. The states having the most outbreaks are given in Table 5.

TABLE 4

Date	Geographical Location	No. of Reports	No. Cases	Deaths
1735–1874	Württemberg	7	920	366
1789–1908..........	Europe	7	1,961	688
1886–1923..........	Germany	3	838	134
1897–1924..........	Prussia	2	397	65
1895–1906..........	Belgium	1	38	3
1901–15............	Denmark	2	4	1
1875–1924..........	France	4	21	3
1860–1926..........	Great Britain	4	75	10

TYPES OF FOOD INVOLVED IN OUTBREAKS OF BOTULISM

In Europe most botulism has followed the eating of preserved meats; but in the United States home-canned vegetables have been responsible for the greatest number of cases. Geiger, Dickson, and Meyer (5) found that, up to 1922, 62.3 per cent of the outbreaks in California were due to home-canned string beans, asparagus, and corn; commercially packed ripe olives and spinach; and home-canned fruits (apricots and pears), while only 24.4 per cent were due to animal products. During 1940, data were collected on 15 outbreaks of botulism, all caused by home-canned goods, in which there were 30 cases and 14 deaths. This information came from newspaper reports, state health department reports, and one report from the United States Food and Drug Administration. The list of foods which have been involved in outbreaks of botulism may be seen in Table 6.

TABLE 5*

DATA COLLECTED BY K. F. MEYER CONCERNING BOTULISM IN THE UNITED STATES AND CANADA, 1899–1947

Location	No. of Outbreaks	Location	No. of Outbreaks
California	179	South Dakota	3
Washington	59	Texas	3
Colorado	30	Wisconsin	3
Oregon	25	Alabama	2
New York	24	Arizona	2
New Mexico	21	Missouri	2
Montana	11	Nevada	2
Nebraska	9	Virginia	2
Tennessee	9	Arkansas	1
Wyoming	8	Florida	1
Idaho	7	Georgia	1
Illinois	6	Iowa	1
Michigan	5	Kentucky	1
North Dakota	5	Maine	1
Ohio	5	Minnesota	1
Canada	4	Mississippi	1
Massachusetts	4	Oklahoma	1
New Jersey	4	Washington, D.C.	1
Pennsylvania	4	West Virginia	1
Utah	4		
Connecticut	3	Total	462
Indiana	3		

* 1,253 cases with 815 deaths, or 65 per cent case fatality rate.

The degree of acidity and the nutrient value of the food for *Cl. botulinum* are the important factors in determining the occurrence of growth of *Cl. botulinum*. The foul, rancid odor in some non-acid foods in which *Cl. botulinum* has grown has not been sufficient-

TABLE 6

FOODS WHICH WERE INVOLVED IN 462 OUTBREAKS OF BOTULISM IN THE UNITED STATES BETWEEN 1899 AND 1947 (2)

Food	No. of Times	Preparation
Vegetables and fruits:		
String beans	97	Three commercially packed
Corn	47	One commercially packed
Beets	24	Two commercially packed
Spinach and chard	22	Ten commercially packed
Asparagus	21	Home-canned
Olives	14	Twelve commercially packed
Peas(?)	10	Home-canned
Figs	10	Home-canned
Beet greens	9	Home-canned
Chili peppers	9	Home-canned
Beans	7	Home-canned
Tomatoes	7	Home-canned
Mushrooms and mushroom sauce	6	Home-canned
Apricots	4	Home-canned
Okra	3	Home-canned
Pears	3	Home-canned
Pickles	3	Home-canned
Vegetables	3	Home-canned
Black-eyed Susan beans	2	Home-canned
Par-par	2	Home-canned
Soybean mash (Natto)	2	Home-canned
Pumpkin	2	Home-canned
Carrots	2	Home-canned
Green peppers	2	Home-canned
Green chili	2	Home-canned
Mixed salad beets(?)	1	
Okra and beans	1	Home-canned
Applesauce	1	Home-canned
Apricot butter	1	Home-canned
Beans and peppers	1	Home-canned
Cauliflower	1	Home-canned
Celery	1	Home-canned
Eggplant and green pepper	1	Home-canned
Mangoes	1	Home-canned
Persimmons	1	Home-canned
Pimiento	1	Home-canned
Okra, sourgrass, tomatoes	1	Home-canned
Squash	1	Home-canned
Succotash	1	Home-canned
Tomato catsup	1	Commercially packed
Tomato juice	1	Home-canned
Tomato relish	1	Home-canned
Turnips	1	Home-canned
Vegetable soup mixture	1	Home-canned
Eggplant	1	Home-canned
Lima beans	1	Home-canned
Pickled beets	1	Home-canned
Pickled string beans	1	Home-canned
Tomato or okra and corn(?)	1	Home-canned
Cucumber	1	Home-canned
Green tomato and green pepper	1	Home-canned
Peaches and pickles	1	Home-canned
Peaches	1	Home-canned
Chili	1	Home-canned
Shallots (Muscari)	1	Commercially packed in Italy

TABLE 6—*Continued*

Food	No. of Times	Preparation
Meat:		
Sausage	6	Two commercially preserved
Ham (deviled ham included)	5	One commercially preserved
Beef	5	Home-preserved
Chicken	3	Home-preserved
Blood sausage	2	Home-preserved
Pork products	2	One commercially packed
Beef tamales	1	Unknown
Buffalo meat	1	Home-canned
Calf's head vinaigrette	1	Home-preserved
Frankfurter sausage	1	Home-preserved
Pork and beans	1	Commercially canned
Liver sausage and blood sausage	1	Home-preserved
Tongue (pickled)	1	Home-preserved
Prepared food(?)	1	Home-canned
Bologna	1	No details
Pickled pigsfeet	1	Home-prepared
Pimiento loaf	1	Home-prepared
Lamb stew	1	Home-canned
Chili con carne	1	Home-prepared
Milk and milk products:		
Cheese and cottage cheese	5	Home-prepared
Milk (canned)	2	Commercially canned
Fish and seafoods:		
Salmon	8	One commercially canned
Tuna	7	Three commercially canned
Clams (clam juice and broth included)	4	Commercially canned
Sardines (1 sardine and tomato sauce)	4	Three commercially canned
Crab	1	Home-canned
Fish (pickled)	1	Home-canned
Herring	1	Home-canned
Sprats	1	Commercially canned
Salmon eggs	1	Home-prepared
Miscellaneous:		
Antipasto (Italian)	1	Commercially packed
Home brew	1	Home-canned
Salad dressing (mustard)	1	Home-canned
Unknown	45	
Total	462	

ly emphasized. Owing to this fact, many foods are probably thrown away and are not eaten. Canned meats, in particular, give off a foul odor as a result of growth of *Cl. botulinum*. With the more acid foods with poorer nutrients for *Cl. botulinum*, the foul odor may not be so noticeable. For example, such is the case in canned string beans.

Recently an outbreak of botulism due to home-canned tomatoes has been reported (8). These tomatoes in a half-gallon jar were opened approximately 20 minutes before the meal. The housewife observed that they had a slightly "off-odor." The sample for bacteriological examination contained juice and seed, with the solid tomatoes more or less disintegrated. The odor was that of butyric acid. In this material Type B toxin was demonstrated by feeding tests on guinea pigs and protection tests with Type B antitoxin. I. C. Hall (9) reported 7 outbreaks of botulism in the Rocky Mountain region for the years 1929–32, one of which was caused by home-canned chili (a condiment sauce made with red peppers, tomatoes, and spices and cooked in vinegar). The chili was spoiled, but no pH studies were reported. For the years 1932–36, Hall (10) reported 14 outbreaks of botulism in the western United States. Chili was found to be responsible for 3 of these outbreaks, and, from 2 of the 3, *Cl. botulinum* was isolated, Type A in one case and Type B in the other. In none of these outbreaks was the pH of the incriminated food reported.

The taste and smell given off by acid food products involved in 9 outbreaks of botulism have been summarized by Slocum, Welch, and Hunter (8). In 3 outbreaks in which home-canned pears were involved, the following changes were noted: In one case the pears were gassy and tasted "sharp," in another the pears had a "peculiar taste," whereas in the third they were abnormal in odor and tasted very acid. Three outbreaks involved home-canned apricots; in the first they were described as tasting "bitter," in the second as having "a peculiar taste," and in the third, "did not taste just right." In one outbreak home-canned tomato-onion chili sauce, which was the product involved, was said to have had an "acrid taste" and to have smelled "strong."

Obviously, many people would not eat food which had a disagreeable odor or a bad taste; but, since there are degrees of tolerance among different individuals regarding the use of spoiled food, it is impossible to rely on spoilage as a safeguard against botulism. Furthermore, evidence of spoilage has not been observed in all outbreaks. The majority of European strains of *Cl. botulinum* and the American Type E strains causing human botulism are nonproteolytic and very likely would give less evidence of spoilage. Often when foods are heated, the volatile products of spoilage are more easily detected than when the food is eaten cold.

Food is frequently eaten by individuals who are aware that it is obviously spoiled. An illustration of this is recorded in an outbreak of botulism reported in South Dakota from home-canned string beans.

> On September 14, 1936, in a small town in the northern part of the state, five people sat down to a meal consisting of potatoes, boiled bologna sausage, home-canned string beans, bread, butter, and coffee. The family consisting of C. A., owner of a small country hotel, his wife, M. A., and three boarders, A. J., J. R. R., and J. S., all sat down together at this meal. The beans, home-canned by the so-called cold-pack method, were noted to be slightly foamy and faintly rancid in odor upon opening the Mason jar [a glass jar with a metal screw top used for canning foods] in which they had been prepared. The landlady questioned their edibility but J. R. R. assured her they would be all right if rinsed off in cold water, whereupon she rinsed them at the kitchen sink through five changes of cold water and served them without further preparation. These beans had been purchased from a truck distributing vegetables from Minnesota and originally offered for sale in the small store attached to the hotel. The remainder had been thriftily processed by the landlady. They were, therefore, wilted and stale at the time of canning. A boarder, J. S., was still able to detect a rancid odor when the beans were passed to him, so he took none of them. He described the odor as being somewhat like limburger cheese, but not strong enough to be offensive. He was the only one who ate none of the beans, although he ate heartily of all the other food; likewise he was the only one of the five to be alive and well 42 hours later [11].

SYMPTOMS OF BOTULISM

A number of different symptoms may result after ingestion of food containing botulinum toxin. In some individuals the symptoms involving the central nervous system, which are characteristic of botulism, are preceded by an acute disgestive disturbance and vomiting. These upsets are not due to peculiarities in the item of food, since many individuals eating the same food will exhibit different reactions. In the outbreak described by Hunter and others (11), of 4 individuals who had eaten contaminated home-canned string beans, only 3 vomited (15–18 hours); the fourth, who had eaten less than a teaspoonful of the beans, had no digestive upset. In an outbreak in Grafton, North Dakota

(12), in which 16 individuals ate home-canned (cold-pack method) mixed vegetables consisting of diced carrots, peas, and cut string beans, 13 died. The 3 who lived had been drinking homemade beer and wine and had vomited before lunch and during or just after lunch. Some of the victims vomited later, but not until after absorption of a fatal dose of botulinum toxin. The author had the opportunity of observing botulism (7) in 2 men. Both ate a lunch containing sandwiches made with canned Italian onions, which had been packed in Naples, Italy. The onions were later shown to contain Type B botulinum toxin. One man died, and the other, who survived, told me that he was nauseated after eating and took a large dose of Epsom salts. He had absorbed enough botulinum toxin, however, to cause diplopia and some difficulty in swallowing. The latter symptom was well illustrated by the patient's statement that he had to drink a pint of coffee to enable him to swallow a doughnut, which was his customary breakfast.

Nausea and vomiting usually come on in less than 24 hours and appear to bear some relation to the degree of spoilage and to the amount of incriminated food consumed. Diarrhea may occur early, but in the later stages of the disease constipation is present. This is illustrated in the outbreaks described by Dickson (3). In outbreak No. 1, 5 persons had eaten spoiled apricots, and none developed nausea or vomiting, owing probably to the feeble growth of *Cl. botulinum* and to the small amount of toxin. In outbreak No. 2, no nausea, diarrhea, or vomiting occurred when only 2 spoonfuls of spoiled home-canned corn were consumed. In outbreak No. 4, a woman opened a jar of home-canned string beans and, noting an unpleasant odor, tasted one of the pods. She developed some nausea, but only after 2 days, at a time when neurological symptoms had developed. In outbreak No. 6, a woman noticed that one of a number of jars of string beans, which she had canned 2 weeks before, was leaking. She tasted one of the pods and noted that it was "slightly sharp." Subsequently, she threw out the contents of the jar. She developed no nausea or vomiting. In all except the first-mentioned outbreak, very small amounts of the food in question were eaten. In contrast to these, in outbreak No. 3, a woman opened a jar of home-canned asparagus and drank the juice. She became nauseated and vomited in about 6 hours. In an outbreak previously referred to, which was reported by Hunter and others (11), the person who ate less than a spoonful of the incriminated string beans had no symptoms of nausea or vomiting, while 3 other adults who had eaten an average helping of the beans became nauseated and vomited.

The typical symptoms of botulism usually appear within 12–36 hours, although as short a period as 2–4 hours is mentioned (5) and a much longer period may elapse. In the outbreak of botulism (12) in Grafton, North Dakota, in which 13 of the 16 persons died, the last person to be affected developed symptoms 108 hours after the meal. During the first 3 days of his illness he went about town, attended the funerals of his fellow-victims, testified before the coroner's hearing, and was interviewed by several doctors, who thought that his symptoms might be psychic in origin (13). He refused hospitalization, but on the fourth day after the onset of symptoms, while running down an alley in response to a volunteer fire call, he fell and was unable to rise. He was picked up by friends and taken to the hospital. At this time he exhibited marked muscular weakness and was unable to turn himself in bed without great difficulty. The sensorium was clear until death, which came from respiratory paralysis. In other words, this patient was active for about 8 days before hospitalization, his symptoms developed slowly, and he did not die until 10 days after ingesting the botulinum toxin.

When gastrointestinal symptoms are present, the patient may complain of burning and distress in the abdomen. Although constipation may be preceded by diarrhea, it often occurs early and is usually present throughout the illness. There is no colic-like pain or tenderness in the abdomen. In the majority of cases the earliest symptom is a peculiar lassitude or fatigue, which is sometimes associated with dizziness or headache and often attributed to constipation.

Such disturbances as double vision, or diplopia, occur early. The third cranial nerve is involved. Blepharoptosis, mydriasis, and loss of reflex to light stimulation are present with the diplopia. Impaired function of the external rectus muscle ap-

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pears. Although there are complaints regarding vision, the difficulty usually involves the extra-ocular muscles and not the retina. Photophobia, nystagmus, and vertigo are occasionally recorded. Difficulty in swallowing occurs early, and difficulty in speech is observed later. Sometimes the throat is described as feeling constricted. The tongue is coated and swollen and moves with difficulty. Paralysis of the pharyngeal muscles occurs in fatal cases, and often there is regurgitation of fluids through the nose and mouth. There is usually considerable dryness of the mouth.

The muscles of the neck are often weakened. Dickson, in outbreak No. 7, quotes a man who was convalescing from botulism as follows: "When putting on my shoes I found it very difficult to get my head and neck back to normal position, and in many cases it was necessary for me to push my head up with my hands." Some patients complain of muscle inco-ordination. This may be due to visual disturbances. Some have difficulty in getting about, and some even experience falls.

There is no retention of urine, although the amount may diminish, since many patients cannot swallow and proper fluid balance is not maintained. The temperature is usually normal or subnormal. When it is elevated, a bronchopneumonia or aspiration pneumonia may have developed. The pulse may be normal but often becomes rapid in the later stages, even though the temperature remains normal. Sometimes the respiration becomes very irregular in the later stages, and a Cheyne-Stokes type has been observed before death. The blood pressure is usually normal, and no abnormalities have been found in the blood cells as regards type or number.

Death is usually due to respiratory failure. The heartbeat has often been detected for some minutes after respiration has ceased. In some cases artificial respiration has sustained life, but only for as long as it was maintained. The Drinker respirator has been used in several cases (1, 14, 15) but not with great success.

One feature of botulism is that the mentality of patients is clear until a short time before death. Although they may not be able to talk, they can often express themselves in writing.

The duration of illness in fatal cases is usually from 3 to 6 days after ingesting the poisonous food. For example, in a series of 173 fatal cases (5), 18 patients died within 48 hours, 117 persons (67.6 per cent) died within 3–6 days after ingesting the poison, and 1 survived for 26 days.

The symptoms produced by all the toxins are similar, despite biochemical differences between strains. The toxins act somewhat like the drug curare upon the end-plates of the nerves (16, 17, 18, 19) and appear to act on the myoneural junction of the skeletal motor apparatus. However, there appears to be a fundamental difference between curare and botulinum toxin, since acetylcholine or nicotine injected intra-arterially still causes contraction of the muscle after botulinum poisoning but not after curare poisoning (20). Small amounts of either Type A or Type B toxins decrease the synthesis of acetylcholine in both *in vivo* and *in vitro* experiments. This decreased acetylcholine synthesis, as far as it is relevant to myoneural function, is suggested as being mainly responsible for the functional paralysis observed in botulism (21). The effect of the toxin may occur proximal to the point at which acetylcholine is produced. The vagus nerve appears to be affected in the same manner as are skeletal nerves. A peripheral circulatory failure is present in botulism. The action of toxin on the nerve endings is not reversed by massive doses of antitoxin.

In a series of 38 guinea pigs (20) the right gastrocnemius muscle was injected with one minimal lethal dose of Type A botulinum toxin. The animals were protected from systemic effects by the administration of 20 units of homologous antitoxin 2 days later. Only 60 per cent recovery of the use of these muscles occurred in 7 months and 90 per cent recovery in 3 animals studied after 1 year. In contrast to this, 100 per cent recovery occurred in 1½ months in a control series of guinea pigs in which the sciatic nerve was crushed. Histological studies of the end-plates in poisoned gastrocnemius muscles in guinea pigs showed bizarre effects, among which was swelling. The long recovery period and degree of irreversibility of botulinum poisoning in the end-plate is in keeping with the lack of success in treating botulism.

TREATMENT

The treatment of botulism is unsatisfactory at best. Obviously, the sooner botulism is recognized, the better is the opportunity for the physician to help those more fortunate individuals who have eaten less of the poisonous food and have not manifested symptoms early. Unfortunately, when the disease is recognized, there is usually no antitoxin available locally. Therefore, more time elapses, during which absorption of toxin and irreparable damage to tissues takes place.

The fact that symptoms may appear in less than 24 hours when potent toxin is given and that antitoxin therapy is of less value once symptoms have appeared is illustrated by some experiments on monkeys by the author (22). As may be seen in Table 7, monkey No. 1 not only showed symptoms early but died in 18 hours as the result of a potent dose of toxin. Other monkeys (Nos. 2, 3, 6, and 9) died or survived, depending on the amount of toxin given. Monkeys Nos. 4, 5, and 10 were all given antitoxin intravenously after symptoms had occurred, but all died.

On the other hand, in one outbreak, which occurred in Ohio, the administration of antitoxin appeared to be of definite value in therapy (23). In Russia, I. M. Velikanov and M. K. Kolesnikova (24) have treated 227 cases of botulism since 1929. Of these, 146 were treated with a specific serum, and 76 were not given serum. The treatment of the remaining 5 cases was not indicated. The mortality in the first group was 18 per cent and in the second group 93 per cent. Analysis of the first group revealed that serum was administered in the agonal stage to several of the 26 who died. Thus, as previously concluded, the effectiveness of the specific serum depended on its early administration, as well as on the dosage. The authors advise a dosage of from 50 to 60 ml., which is to be repeated within 5–10 hours if no improvement is noted. They found that the serum is best given intramuscularly. In 2 apparently hopeless cases the intraspinal introduction of 20 ml. of the serum had a remarkable effect. These authors conclude that specific serum therapy in botulism gives the best curative result.

TABLE 7

EFFECT OF BOTULINUM TYPE A TOXIN AND HOMOLOGOUS ANTITOXIN ON MONKEYS

Monkey Number	Wt. in Kg.	Ml. of Toxin Fed	Number of M.L.D. (Mouse)	Hours Elapsing before Symptoms Appeared	Toxin in Blood	Ml. of Antitoxin*	Time of Death after Feeding (Hours)	Comments
1....	.....	2	400,000	17.5	+	0	18	No toxin found in stomach but was demonstrated in large intestine in 1–2,000 dilution
2....	.....	0.1	20,000	22.75	+	0	24	Ate hearty meal 20.5 hours after feeding; no toxin found in the stomach
3....	3.5	1	2,000+	45	0	0	51	No toxin demonstrated in stomach, small intestine, or large intestine
4....	3.0	1	2,000	102	......	3(IV) 1(SC)	124	Given 20 ml. of 10 per cent glucose intraperitoneally after 121 hours
5....	2.6	2	20,000	53.5	0	2(IV)†	62	Fed 100 ml. cream with toxin; given 0.25 gm. barbital by mouth after 55.5 hours; given 0.25 gm. barbital intravenously after 57 hours
6....	3.5	2	20,000(?)	71	0	0	73.25	Given 0.18 gm. barbital intravenously after 73 hours
7....	.....	0.1	200+	0	......	0	Lived	Blood was tested for antitoxin 32 days later; the mouse receiving 1 ml. serum plus one M.L.D. of toxin died after a longer time than did the control mouse, which received the toxin alone
7....	.....	5	3,500	Not observed	+	0	38.5	Fed 50 days after previous feeding
8....	2.6	0.01	20+	0	......	0	Lived	No symptoms
8....	.....	1	10,000	....	......	....	Lived	Fed 14 days after first feeding; showed symptoms which consisted of general weakness; blood was tested for antitoxin 18 days later; the mouse receiving 1 ml. of serum plus one M.L.D. of toxin lived a day longer than did control mouse
8....	2.9	0.1	200+	0	......	0	Lived	Fed 75 days after last feeding; toxin 10 days old
8....	2.9	2	40,000	19	+	0	27.5	Fed 4 days after last feeding; first symptom consisted of general weakness; considerable effort was required for the animal to move
9....	3.5	0.1	200+	0	......	0	Lived	No symptoms
9....	.....	2	40,000	Not observed	+	0	18	Fed 4 days after last feeding
10....	3.9	2	40,000	19.5	+	3(IV)	25	Fed 7.5 gm. $MgSO_4$ in water 8 hours after feeding toxin; part of the $MgSO_4$ was vomited, and 5 more gm. were fed; 20.5 hours after feeding toxin, the monkey was fed 100 ml. of strong coffee; after taking 5 ml. of blood for toxin titration, antitoxin was given

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* The antitoxin was given when symptoms appeared. (IV) = intravenous; (SC) = subcutaneous.

† This antitoxin was given 2.5 hours after symptoms appeared.

Since botulinum antitoxin is the only known specific therapeutic agent for botulism, its use should be encouraged, even though the disease is advanced. Moreover, although botulinum toxin is not formed in toxic food after ingestion, damage to the body may continue as the result of a gradual absorption of toxin as long as the latter remains in the intestinal tract. The author would recommend that large doses of polyvalent antitoxin be given when the specific type of organism is unknown. In the therapy of botulism, at least 50,000 units should be given intramuscularly after the patient has been tested for hypersensitiveness and, if necessary, desensitized. W. E. Watson (25) recommends giving the antitoxin intravenously in large doses in 1,000–1,500 ml. of 5 per cent glucose in normal salt solution with 8 minims of epinephrin added. When small amounts of toxin are ingested because of laboratory accidents in pipetting, 5,000 units of the antitoxin may be given. In the author's experience, two such accidental cases have been so treated with no untoward effects. In addition, enemas are recommended for washing out the colon to get rid of any toxin which might be retained because of constipation.

In addition to specific treatment with antitoxin, it is important that other measures be taken. When symptoms of botulism develop, symptomatic treatment is directed chiefly to the maintenance of the fluid balance of the body. Pharyngeal paralysis, frequently an early symptom, necessitates the administration of fluids parenterally. Even though the patient may be willing to attempt to take fluids or food by mouth, this is best discouraged because of the danger of aspiration pneumonia. Measures should also be taken to avoid the aspiration of saliva. The patient should be kept in quiet, restful surroundings and encouraged to avoid even the slightest unnecessary muscular exertion.

In outbreaks of botulism in which several individuals have consumed food containing toxin, those who may have only tasted the food or eaten sparingly of it usually do not develop symptoms until considerably later than those who have eaten generously of the poisonous food. Antitoxin should prove of great prophylactic value to such patients. There is considerable experimental evidence in favor of such prophylactic treatment.

In addition to the effect of dosage in determining the time of onset of symptoms, there may be another factor to consider in explaining delayed symptoms. Gastrointestinal upsets which occur early in some patients may cause the toxin to remain only a short time in the stomach and small intestine. Once the toxin is in the colon, it may remain for some time, since constipation is the rule in many cases. Under such circumstances of a long stay in the colon there may be slow and gradual absorption until a sufficient quantity is absorbed to produce symptoms. This contention is borne out by experimental work with Type A botulinum toxin in the monkey (26) and is further supported by the fact that botulinum toxin is not readily destroyed in intestinal contents. Thus toxin has been found to persist in isolated loops of ileum in the dog (27) for at least 12 days.

Like man, the guinea pig is highly susceptible to botulinum toxin. The following experiment (28) suggests that the greatest absorption of the toxin probably occurs in the upper part of the intestinal tract. Two guinea pigs were given an *intracecal* injection of 1 ml. of Type A botulinum toxin, of which 0.001 ml. constituted a minimum lethal dose for mice injected intraperitoneally. One animal died on the seventeenth day, with typical symptoms of botulism, whereas the other guinea pig survived. Seventeen days later the animal which survived was *fed* the same amount of toxin (1 ml.) of the same lot, which, in the interim, had been stored in the refrigerator. This latter animal developed symptoms of botulism on the fourth day and died on the seventh day.

MORTALITY AND PATHOLOGY

The mortality from botulism is approximately 65 per cent in the United States but is low in Europe. In many outbreaks all who eat the incriminated food die. In other instances some escape. Death hinges on the amount of botulinum toxin ingested and absorbed. When a large dose is taken, the onset of symptoms occurs in a short time. According to Geiger and others (5), symptoms occurred within 48 hours after ingestion of the poison in 85 per cent of 173 fatal cases. Although vomiting shortly after eating may get rid of the toxin, it does not usually occur for several hours. During the lapse of this time a fatal dose of botulinum toxin is often absorbed.

There are no gross lesions which characterize botulism after death. When death occurs in less than 48 hours, microscopic changes are often not observed. In patients in whom the progress of the intoxication has not been too rapid, cellular thrombi have been found (5) in the meningeal veins. The thrombi consist of dense masses of fibrin arranged in thick bands, with many polymorphonuclear leukocytes enmeshed between the strands (3). Although the thrombi when present may suggest that death was caused by botulism, they cannot be considered pathognomonic of botulism (5).

LABORATORY DIAGNOSIS OF BOTULISM

The laboratory diagnosis of botulism is not always easy, as the suspected food may have been discarded and necropsy material be all that is obtainable. Sometimes the food has been fed to chickens, which have subsequently developed limber-neck (fowl botulism) and died. In fact, in studying outbreaks of botulism, it is always well to inquire whether or not items of food have been fed to chickens. If so, were the chickens made ill, or did any of them die? It has been reported (29) that chickens, although susceptible to Type A toxin when ingested, are highly refractory to Type B toxin administered by the same route. However, in a subsequent report by other investigators (5) chickens were reported killed by eating food containing Type B toxin. A sample of the food suspected of causing botulism may be simply a small portion remaining in a container or a few dried fragments rescued from the garbage; it may be vomitus, stomach washings, or portions of the bowel taken at necropsy; it may be another can from the same pack of canned food. In the case of the onions (7) which were canned in Naples, Italy, many unopened cans from the same lot contained viable *Cl. botulinum* Type B, as well as potent toxin. On the other hand, in the Loch Maree tragedy in the western Highlands of Scotland, in which eight people died who ate potted wild-duck paste (30), over a million jars had been packed that year, and apparently only one contained *Cl. botulinum* organisms and toxin.

Strains of Type A and Type B *Cl. botulinum* often produce gas and give rise to "swells" in food packed in tin cans, but not invariably. A systematic bacteriologic and toxicologic study was made (31) on 346 cans of commercially packed food products of both plant and animal origin. These were artificially inoculated with washed and heated spores of *Cl. botulinum* and incubated at room temperature and at 35° C. or at both these temperatures for periods extending from 10 days to 12 months. From these studies corn, peas, salmon, sweet potatoes, and pumpkin were found to undergo spoilage quickly and developed very toxic decomposition products. Asparagus and beets became spoiled and toxic without swelling, and the spoilage was not sufficient to warn the consumer. Growth and toxin production of *Cl. botulinum* were found to be irregular in spinach; when growth occurred, it might or might not give rise to "swells." In some spinach a spoiled, butyric acid or cheesy odor was evident. Bulging occurred in 50 per cent of the inoculated cans of string beans. Toxins have been frequently found in cans which have a normal odor and contain firm pods of a natural color.

In the laboratory diagnosis one cannot rely on the odor or gross signs of spoilage in previously unopened containers as evidence of the presence of toxin. It is a good plan to determine the pH of the product in question, because much can often be learned from such data in regard to factors limiting growth of the organism. Growth is uncertain at pH 5.4 or lower, although it depends somewhat on the nutritive value of the food to *Cl. botulinum*. The glass-electrode potentiometer is of value in making determinations in samples of food that are highly turbid or colored.

Obviously, the presence or absence of toxin in a specimen submitted for examination is of great importance. The presence of toxin is determined as follows: The sample is ground in a mortar with sterile sand and saline as needed. The suspension is centrifugalized at high speed for 1 or 2 hours. One-half-ml. portions of the supernatant fluid are then withdrawn and injected intraperitoneally into mice, some of the mice being protected, respectively, with Types A and B antitoxins. If all animals should die with symptoms simulating botulism, Type E antitoxin should be used in a second series of tests, since outbreaks of this type have been described in man. Filtration through a Berkefeld filter is purposely avoided, inasmuch as it reduces the toxicity of the toxin; but, if the sample is large and known to be badly contaminated, filtration may be carried out as an additional control. Unfiltered material may be also fed to guinea pigs.

Methods of culturing *Cl. botulinum* have been described (32), and the demonstration of *Cl. botulinum* by culture has been resorted to in some outbreaks. One must be very cautious in interpreting such data, since *Cl. botulinum* is ubiquitous and may be a contaminant of any specimen.

The identification of the botulinum toxin is of greatest value. Sometimes it may be demonstrated in necropsy material from the intestinal tract. It can also be found in serum, as first ascertained by M. Kob (7, 33, 34). Recently the literature on the demonstration of botulinum toxin in the blood and tissues of patients has been reviewed (35). Obviously, it is important to test the serum of fatal cases for toxin by the intraperitoneal injection of mice in the laboratory diagnosis of botulism, especially when samples of food are not to be had. Moreover, in serum samples, the gross contamination, such as is encountered in specimens from the bowel, is avoided.

The determination of types is established by finding the type of antitoxin which neutralizes the toxin to be tested. As mentioned before, the symptoms in the mouse, injected intraperitoneally with the food, food extract, or supernatant fluid from a culture, are the same for all types of botulinum toxin. Serological tests for typing these organisms are not practical, since the groups are heterogeneous (4, 36, 37), and each antitoxin type has several antigenically distinct agglutinative and complement-fixing groups. For this reason it is time-saving to identify them simply on a toxigenic basis.

CONTROL OF BOTULISM

The control of botulism requires a knowledge of the bacteria which are responsible for it, in particular their habitat in nature, the reason for their growth in preserved foods, and the properties of their toxins.

The organism.—*Clostridium botulinum* is a name applied to a group of rod-shaped, spore-forming, anaerobic bacteria producing exotoxins which may be absorbed from the intestinal tract and which give rise to a common train of symptoms. Five types have been distinguished on the basis of the specific nature of their toxins. These are designated as *Cl. botulinum* or *parabotulinum*, Types A, B, C, D, and E. The proteolytic and cultural properties (38, 39) are used by some workers in designating the species as *botulinum* (nonproteolytic) or *parabotulinum* (proteolytic). Animals immunized with a toxin of a specific type produce antitoxin in their serum for only that type of toxin. The toxin of one type is, therefore, not neutralized by the antitoxin of a heterologous type. In general, the toxin-antitoxin relationships are constant, but occasionally they vary. For example, a strain of *Cl. botulinum* from Australia resembled Type C *Cl. botulinum*, and the toxin produced by this strain could be neutralized by Type C antitoxin. The converse, however, was not true, since the antitoxin produced against the Australian toxin would neutralize the Australian toxin but not the toxin produced by Type C cultures (40). Human botulism has been reported for Types A, B, and, more recently, E. Types C and D have not been reported as causing human outbreaks of botulism.

Type A and, to a certain extent, Type B of *Cl. botulinum* are proteolytic and digest coagulated egg white, whereas Types C, D, and E do not. Gelatin is liquefied by strains of all types. Many of the European Type B strains are not proteolytic (41).

In general, the cells of all the types are large gram-positive rods. The spores are usually subterminal but are terminal in Type C *Cl. botulinum*. The vegetative cells contain flagella (peritrichate) and are motile under suitable anaerobic conditions.

The biochemical reactions of strains of *Cl. botulinum* vary with reference to certain test substances, as may be seen in Table 8. All strains ferment dextrose, levulose, maltose, and glycerol; but there is considerable variation within the Type A and Type B strains with reference to the other test substances. Comparatively few strains of Types C, D, and E have been tested. Type D strains produce acid only in fermentable test substances, which characteristic separates them from all others.

Clostridium botulinum in the soil.—Since many different types of food of both plant and animal origin have been involved in outbreaks of botulism, the natural habitat of *Cl. botulinum* was thought to be the soil. A careful study of soils from the United States and from many other parts of the world has been made at the Hooper Foundation, University of California (42*a–g*). A less extensive study was made at the University of Chicago under the direction of the late Professor E. O. Jordan, but the results were never reported. Meyer and Dubovsky (42*c*) made an analysis of 1,538 samples of soil, vegetables, feed, and manure collected from every state of the United States except Virginia. They concluded that *Cl. botulinum* is a common soil anaerobe of the western states of the Cordilleran system, is less frequently encountered in the Atlantic states, and is relatively rare in the

TABLE 8

FERMENTATION REACTIONS OF VARIOUS TYPES OF *Cl. botulinum* AND *Cl. sporogenes*

Type	Dextrose	Levulose	Maltose	Glycerol	Dextrin	Salicin
A...........	AG[*]	AG	AG	AG	AG[†]	AG[†]
B...........	AG	AG	AG	AG	AG	AG[†]
C[‡]..........	AG	AG	AG	AG	AG	O[§]
D...........	A[||]	A	A	A	O	O
E...........	AG	AG	AG	AG	AG	AG
Cl. sporogenes	AG	AG	AG	AG	O	O

Type	Galactose	Inositol	Adonitol	Sucrose	Lactose	Raffinose, Inulin, Dulcitol, and Mannitol
A...........	O[¶]	O[¶]	O	O	O	O
B...........	O	O[¶]	O[¶]	O[¶]	O	O
C[‡]..........	AG	AG	O	O	O	O
D...........	A	A	O	A	A	O
E...........	....	AG	AG	AG	....	....
Cl. sporogenes.	O	O	O	O	O	O

[*] AG = Acid and gas produced.

[†] Some strains do not ferment.

[‡] One C strain from Australia fails to ferment in all tests.

[§] O = No acid or gas is produced.

[||] A = Acid only produced.

[¶] An occasional strain may ferment.

middle western states, the Great Plains, and the Mississippi Valley. They found that *Cl. botulinum* is more prevalent in virgin soils than in dirt, soil, or manure collected from animal corrals, pigpens, etc. Vegetables, fruits, and feeds are frequently contaminated with the spores of *Cl. botulinum.* String-bean pods and leaves, moldy hay, ensilage, and decayed vegetation may yield a relatively high percentage of positive cultures. In fact, these authors conclude that *Cl. botulinus* is ubiquitous and may be found anywhere and at any time. Botulinum organisms were not found (42*b*) in land soil of the Aleutian archipelago but were found in moraine, glacier, and mountain soil collected around Lake Louise in the Canadian Rockies. Soil samples from provinces (42*b*) of Prince Edward Island, Nova Scotia, Quebec, Ontario, and British Columbia have contained *Cl. botulinum.*

Type B *Cl. botulinum* has been demonstrated in soil and vegetable specimens collected in Belgium, Denmark, England, the Netherlands, and Switzerland (42*e*). It has also been found in soil from the island of Oahu, in the Territory of Hawaii, and from the provinces of Chilili and Shansi, in China (42*g*). Type A has been found in two Hawaiian and in one Chinese soil specimen.

From the work reported it is obvious that *Cl. botulinum* is widespread in the soil. Meyer and Dubovsky make the following statements:

> The soil of the Western States, inclusive of the Great Plains, yield, mainly, *B. botulinus*, Type A, while the Mississippi Valley and Great Lakes region is characterized by a striking predominance of Type B. Similarly prevalent is this latter type in the Atlantic States of Maryland, Delaware, New Jersey, Georgia, and South Carolina, while scattered findings of Type A in Maine, New York and Pennsylvania indicate the existence of breeding places in virgin forests and mountains. Soils which are subjected to intensive cultivation and fertilization contain, as a rule, *B. botulinus* [= *Cl. botulinum*], Type B [42*d*].

Out of a total of 2,162 specimens examined (42*d*), 26.8 per cent contained toxic cultures and 18.2 per cent were typed. This does not mean that nearly three-quarters of the specimens were free of *Cl. botulinum*, because the method of detecting *Cl. botulinum* in soil does not preclude many other bacterial species which survive the heat treatment and appear as contaminants. It consists of heating the specimen to destroy many of the less heat-resistant bacteria. The growth of *Cl. botulinum* is demonstrated indirectly by injecting some of the centrifugalized supernatant fluid from the culture into a test animal, either a guinea pig or, more usually, a white mouse. If the animal dies within 4 days, an attempt is made to determine whether or not the toxin is that of *Cl. botulinum* and, if so, to determine the type. This test is usually done by injecting three mice intraperitoneally with the same dose that had previously proved fatal (usually 0.5 ml.). Of these, one serves as a control, one is protected with Type A antitoxin, and the other with Type B. Other organisms, which survive the heat treatment, however, may grow and inhibit the growth of *Cl. botulinum* or destroy botulinum toxin which has been formed. Thus, in some soil samples which the author has tested for the presence of *Cl. botulinum*, a toxin was demonstrated which was lethal to mice in 4 days after they had been injected intraperitoneally with 0.5 ml. of the centrifugalized supernatant fluid from the culture. Subsequently, upon attempting to type the toxin, none of the mice receiving the material died. The organism which was found responsible for destroying the toxin was *Cl. sporogenes* (43). When mixtures of pure cultures of *Cl. sporogenes* and *Cl. botulinum* were made, *Cl. sporogenes* often overgrew *Cl. botulinum* and subsequently destroyed some of the toxin (44). Dubovsky and Meyer (42*a*) observed that "whereas small quantities (10 gm.) of manured soil furnish toxic cultures, 50- or 100-gm. specimens reveal no demonstrable toxin when treated under identical conditions."

These observations make it clear that a negative laboratory test for *Cl. botulinum* does not mean the absence of *Cl. botulinum* in the soil specimen. Furthermore, the demonstration of toxin in over one-fourth of the soils tested indicates that nowhere in nature can soil be considered to be free from the spores of *Cl. botulinum*. Since the demonstration of the toxin in soil cultures depends in part upon the size of the inoculum (43), it is conceivable that botulism may result from underprocessed food in an area where no *Cl. botulinum* was demonstrated in soil cultures. Certainly, the amount of soil necessary to contaminate vegetables would be less than the amount of soil used for the detection of the organism in a survey sample.

Effect of moisture and sodium chloride on the growth and toxin production of Cl. botulinum.—In dehydrated meat (45) experimentally inoculated with the spores of Type A *Cl. botulinum*, toxin developed when sufficient water was added to make the moisture content more than 30 per cent.

Jensen (46) states that 3–3.5 per cent salt in cured canned pork and beef meats prevents germination and growth of heavy inocula of spores of aerobic and anaerobic thermophiles.

When cured canned meats containing 3.5 per cent sodium chloride, seeded with *Cl. sporogenes* No. 3679 (NCA) and processed to F_0 1.0, are incubated for two years at 99° F., no growth or spoilage takes place (47). A comprehensive test was conducted with spores of *Cl. botulinum*, Type B No. 213 (48). Thirty thousand to 35,000 spores per gram and 5,000,000 spores per gram were inoculated into two lots of liver sausage (50 per cent moisture) containing 2.78 per cent salt and 3.6 per cent salt, canned in 14-gm. thermal death-time cans, and retorted at 235° F. for 4 minutes and at 2-minute intervals thereafter to 16 minutes. The test lots were incubated at 98° F., and cans were examined for viable spores and toxin at intervals for 1½ years. None of the lots (4–16 minutes) showed growth or toxin formations. Spores were viable up to 1 year. In one test under these conditions, when 30 cans were retorted for 2 minutes only, two cans showed growth and toxin in 4 months (48).

A comprehensive test was made with sliced bacon seeded with spores of *Cl. botulinum*, Type A (49). The slices containing salt (1, 1.25, 1.5, 2, and 2.5 per cent) were placed in vacuum-sealed packages and incubated at 45°, 72°, and 98° F. and were examined for toxin at intervals of from 4 days to 6 months. No toxicity resulted below 72° F. and brine concentrations above 6.5 (1.5 per cent sodium chloride). Identical tests were conducted with vacuum-packed sliced dried beef (10.9–15.7 per cent brine*). No toxin developed under these conditions. Country-style cured hams containing 7.7–16.8 per cent brine were also nontoxic

[*] Brine concentrations are calculated as follows: Canned pork luncheon meat, with 57 per cent moisture and 3.5 per cent sodium chloride = 5.78 per cent brine; liver sausage, with 50 per cent moisture and 3.5 per cent sodium chloride = 6.9 per cent brine; divide the salt content by the sum of moisture and salt and multiply by 100 = Per cent brine.

when tested in this manner (49). The precise effect of spores of *Cl. botulinum* in meat are detailed elsewhere (50).

Resistance of the spores of Cl. botulinum to heat.—Since outbreaks of botulism occur from eating certain inadequately heat-preserved foods, the problem of determining the heat resistance of the spores of *Cl. botulinum* is of paramount importance. In fact, heating is a selective process which kills less heat-resistant organisms and permits more heat-resistant types to survive and grow without having to compete with these other forms. Unfortunately, the spores of *Cl. botulinum* are among the more heat-resistant types of microörganisms.

Spores in the dry state are very resistant to heat. Old cultures of *Cl. botulinum*, Type B (51), in brain medium were mixed thoroughly and swabbed on the inside of sterile culture tubes and dried. After drying they were heated at different temperatures in a Lautenschlager-type gas oven. Tubes were removed at 5-minute intervals, and sterile, freshly boiled dextrose broth was added, after which a sterile paraffin seal was made to keep conditions anaerobic. There was some variation in the heat resistance of five strains tested. At 110° C. the time of survival averaged more than 120 minutes. At 140° C. the variation was between 15 and 60 minutes. At 160° and 180° C. the time of survival was between 5 and 15 minutes.

The resistance of *Cl. botulinum* to heat in a liquid substrate appears to differ with the number of spores present. When very small numbers (9 or 10 spores) are present (52), they are destroyed within 10 minutes at 100° C., whereas larger numbers (53, 54) are more heat-resistant. Different strains, however, vary in this respect. Thus certain strains appear far more heat-resistant with few spores present than do other strains with large numbers of spores present (42*e*). Esty and Meyer (54) studied the heat resistance of 109 strains of *Cl. botulinum* (78 Type A, 30 Type B, and 1 nontoxic) from various sources. They found the heat resistance to vary from 3 to 80 minutes at 105° C. The spores which they used came from cultures in pea–peptic-digest broth with a pH of 8.0 and were heated in a phosphate solution of pH 7.00–7.12. The suspensions heated contained several billions of spores in some cases. Under these conditions they found that the maximum heat resistance of the spores of *Cl. botulinum* is as shown in the accompanying table.

4 min.	at 120° C.	(248° F.)
10 min.	at 115°	(239°)
32 min.	at 110°	(230°)
100 min.	at 105°	(221°)
330 min.	at 100°	(212°)

Esty (54) suggested time-temperature relationships for completely destroying all spores of *Cl. botulinum.*

100° C.	360 min.	115° C.	12 min.
105°	120	120°	4
110°	36		

The heat resistance of the spores of Type C *Cl. botulinum* (55) is considerably less than for Types A and B. They resist heating to 100° C. for 60 minutes but not for 90 minutes. Spores of Type D (56), produced in peptic-digest gelatin medium, incubated at 37° C. for 6 days, and diluted to approximately 4,000,000 spores per milliliter in phosphate solution, survived boiling for 100 minutes but were destroyed after 120 minutes. Spores of Type E (57) resist a temperature of 80° C. for 10 minutes. Other strains of Type E have been tested (58). A suspension of 5,000,000 spores per milliliter in buffer solution (pH 7.4) failed to show growth after 5 minutes at 100° C. or after 40 minutes at 80° C. In spite of the low heat resistance of Type E, human outbreaks have been reported that were due to German canned sprats, smoked salmon coming from Nova Scotia (59), and canned mushroom sauce (1).

Young spores have been found more heat-resistant than old ones. When the heat resistance of dried spores was tested in dry heat (51), a 10-day suspension of spores was found much more heat-resistant than a 90-day suspension. In one experiment (53) a strain of *Cl. botulinum* was grown in pea–peptic-digest broth with and without gelatin and in neutral grain medium at 37° and 28° C. Tests of resistance were made from 1½ through 186 days. Maximum resistance was obtained in 1½–3½ days in pea gelatin, 2½ days in pea–peptic-digest, and 6–16 days in grain medium at 37° C. After these periods, resistance rapidly decreased, although the number of spores present in the medium remained fairly constant.

Sodium chloride in concentrations of 8, 10, and 20 per cent solutions greatly decreases the heat resistance of the spores, whereas solutions of from 0.5 to 1.0 per cent concentration appear to enhance it. The heat resistance of spores of *Cl. botulinum* in the juices of 17 varieties (53) of canned food shows a variation of from less than 10 minutes to 230 minutes at 100° C.

Spores of *Cl. botulinum*, Types A and B, were found to have greater thermal death times when the recovery cultures were incubated at temperatures of 24°–27° C. than when incubated at 31° or 37° C. (60).

There may be a marked delay in the germination of spores of *Cl. botulinum* after they have been heated. Dickson and his coworkers (61) observed that some tubes of medium containing small numbers of heated spores would remain apparently sterile for weeks or months after others had shown growth but would eventually show vigorous growth with toxin formation. Appearance of growth after 72 months has been reported (62).

The problem of heat penetration is extremely important when dealing with foods. In large gallon containers, it is difficult to heat certain products so that the center of the cans will contain no viable *Cl. botulinum* without impairing the product. This problem is well understood by the commercial canner. For example, each specific item of food is tested in the laboratory before processing times and temperatures are recommended. Of these various tests (63), one is concerned with the rate of heat penetration in different containers under laboratory and factory conditions.

The foregoing studies on the heat resistance of the spores of *Cl. botulinum* should convince everyone that home canning by the cold-pack method is extremely dangerous. Nevertheless, this method of canning is still widely used in the United States. It is to be hoped that the advice constantly given by schools, the press, and the radio will have its effect on those now canning by the cold-pack method so that they will change to the pressure-cooker method.

Botulism may be prevented if sufficient care is given to home canning. It is important that fresh and firm fruits and vegetables be thoroughly washed and cleaned and canned as soon as possible after picking or gathering. A nonacid food, such as vegetable, meat, poultry, or fish, should be processed in a pressure cooker for a definite period of time (64), with an accurate gauge or thermometer. However, if canning is done by any other method, the food should be reboiled for 15 minutes before tasting or eating, counting time after boiling has begun. A canned food having a disagreeable odor or showing gas pressure should never be tasted. Such spoiled food should be treated by adding several spoonfuls of lye to the can or jar. The can should then stand for not less than 24 hours, after which it should be buried. A glass container should be carefully soaked in a hot lye solution after the spoiled food has been destroyed.

Other factors affecting the growth of Cl. botulinum.—The germination of spores of *Cl. botulinum* requires a higher temperature of incubation than for growth and multiplication alone. Growth and toxin production occur within a few days at 20° C., when either vegetative cells or spores are inoculated. When vegetative cells are inoculated into suitable media held at 15° C., toxin is produced, but heat-detoxified spores rarely produce toxin at this temperature. Foods stored at room temperature or under slight refrigeration may become dangerous fairly rapidly, but when kept at less than 10° C. will remain safe for a considerable length of time (65). *Clostridium botulinum* may sometimes develop and produce toxin in frozen foods which have been allowed to thaw and have been stored at temperatures of 10° C. or higher. However, if properly handled and kept frozen until used, frozen foods are probably as safe and satisfactory as fresh foods (66).

The germination of spores of *Cl. botulinum* has been found to be affected by minute amounts of substances in the general category of impurities (67). Such impurities were stated to occur in all organic media and possibly in tap water. When soluble starch was incorporated in the media used for testing the germination of spores, more uniform results occurred between replicate tube counts of a given dilution. The starch was thought to have an adsorption effect on the impurities. The germination process of spores of one strain of *Cl. botulinum*, 62A, was found by the same workers (68) to be logarithmic. In air atmosphere the lag period in germination varied inversely with the logarithm of the number of spores per milliliter in the inoculum.

Beets and carrots canned in plain unlacquered cans were found not to support the growth (69) of either Type A or Type B *Cl. botulinum* experimentally inoculated into the cans. In similar experiments with lacquered cans, growth and toxin production occurred. The addition of a soluble tin citrate complex to each vegetable produced inhibition of growth in both lacquered cans and test tubes. Concentrations of tin required to prevent growth were approximately 150 p.p.m. in beets and 30–60 p.p.m. in carrots. This study was extended to include nine additional vegetables (70). Inhibition of growth at 20°, 30°, and 37° C. for periods up to 6 months was observed in plain cans of French-type beans (cut green beans), silver beets (Swiss chard), and parsnips, in addition to beets and carrots. No inhibition was observed in plain cans of asparagus, cabbage, cauliflower, peas, potatoes, and white turnips. Inhibition of growth in plain cans was bacteriostatic and was associated with concentrations of dissolved tin which exceeded the values for lacquered cans. The concentration of tin in solution found necessary to prevent growth differed considerably between vegetables and was positively correlated with reported protein contents. Under no circumstances should dissolved tin in a product be relied upon to replace safe processing of canned goods.

Pathogenicity of Cl. botulinum.—Outbreaks of human botulism are caused principally by the exotoxins of Type A and Type B *Cl. botulinum.* A few have been caused by the exotoxins of Type E. Type D, isolated from toxic carrion in South Africa, produces *lamziekte*, a fatal cattle disease in Bechuanaland, Orange Free State, and in the northwest portion of the Cape Province. The disease *lamziekte* was renamed "parabotulinum" when the organism isolated from the specimens in question by Theiler and his associates (71) was named by them "*Clostridium parabotulinum bovis.*" In the same year Theiler and Robinson (72, 73) described an organism of the *parabotulinum* group which was isolated from the carcass of a dead rat found in a haystack near a stable. This organism was supposedly connected with spinal paralysis of mules or equine parabotulism. The biochemical reactions of Type D *Cl. botulinum* are listed in Table 8. Small doses of toxin appear to be fatal to ruminants *per os*, especially to goats and cattle, but not to laboratory rodents and apes (56). In fact, the fatal feeding dose is at least two hundred to three thousand times that of the inoculating dose. The susceptibility of the guinea pig, mouse, and rabbit to subcutaneous injections of Type D toxin (56) is about the same as for the Types A, B, and C. One hundred thousand guinea-pig M.L.D.'s of the toxin are without effect when injected subcutaneously into a chicken. The monkey is resistant to Type D toxin given orally in doses of 40,000 and 200,000 guinea-pig M.L.D.'s. Whether man is susceptible to Type D toxin *per os* is not known; but, since no human cases have been reported, man may be as resistant as the monkey is. The chicken is resistant to subcutaneous injections of toxin of Type B *Cl. botulinum*.

Type E *Cl. botulinum* produces a toxin of such potency (58) that a subcutaneous injection of 0.02 ml. is an M.L.D. for a 350-gm. guinea pig. Characteristic symptoms of botulism have followed the subcutaneous injection of the toxin into guinea pigs, mice, rabbits, and kittens (74). Similar symptoms develop when monkeys, guinea pigs, and mice are fed the toxin, whereas rabbits are resistant when fed. The toxins produced by two differents strains of Type E *Cl. botulinum* (74), although reciprocally neutralized by their antitoxins, differ in their effect when subcutaneously injected into White Leghorn chickens. With one toxin (8) guinea-pig M.L.D.'s gave rise to fatal botulism, whereas doses of the other toxin several hundred times as large failed to kill or induce symptoms of botulism.

Type C *Cl. botulinum* has not been reported as responsible for human botulism. Bengtson (75) first isolated this organism from fly larvae and showed that the toxin was not neutralized by antitoxin for Types A and B. Kalmbach (76) made a study of so-called "duck sickness." In 1910 untold thousands of waterfowl perished in the marshes about Great Salt Lake, Utah; and the years immediately following saw a recurrence of the sickness to a less extent. The bodies of nearly fifty thousand ducks were buried at the mouth of Bear River, Utah, from September 7 to

September 26, 1913. Kalmbach reproduced the disease in ducks by feeding body tissues from birds dying of duck sickness which had been kept at a temperature of 85° F. for 5 or more days. L. T. Giltner and J. F. Couch (77) isolated *Cl. botulinum*, Type C, from mud and water from an infected area in Tule Lake, California, while an outbreak of duck sickness was at its height. Furthermore, *Cl. botulinum*, Type C, has been cultured from the tissues of wild mallards, pintails, and ring-billed gulls that had died of, or were killed while afflicted with, duck sickness.

Duck sickness is widespread in nature. It has been reported in the Province of Alberta, Canada, about 45 miles east of the city of Calgary and 130 miles north of the Montana border (78). In the summer of 1940 several hundred wild ducks died in a bird sanctuary in Jackson Park, Chicago, while in Lincoln Park, only a few miles north, no duck sickness was evident. Maggots and livers taken from some of the dead ducks were studied in the author's laboratory by Dr. Ellen Davison. The maggots and liver were ground separately in sterile mortars with sterile sand and saline solution. The samples were centrifugalized to rid them of the gross particles and then filtered through Berkefeld filters. Toxin was demonstrated in the liver and in the filtrate of the maggot extract. This toxin was neutralized by Type C *Cl. botulinum* antitoxin. Toxicity tests were made by inoculating white mice subcutaneously and making appropriate toxin controls. Duck sickness occurs during the hot months of the year in stagnant waters but apparently not in large bodies of water. The toxin evidently continues to be formed after the death of the animal and thereby contributes to the toxicity of the water.

There are many problems regarding Type C botulism which are unsolved. Much remains to be learned regarding the behavior of the organism in nature. The abundance of the organisms in the tissues after death suggests either an ante mortem spread of the organism or a rapid post mortem invasion of the tissues and a predominance over other naturally occurring microörganisms. Type C *Cl. botulinum* is abundant in nature over a wide geographic area, and it is a mystery why no cases are reported in man. The rhesus monkey (79) is resistant to the toxin of Types C and D *Cl. botulinum* when they are given orally, and such may be the case for man.

The problem arises as to the danger of botulism from the ingestion of large numbers of organisms. Suppose, for example, that a preserved food contains a large number of the incriminating organisms and that in its preparation for consumption it is sufficiently heated to destroy the toxin but not the spores. Could botulism follow? G. E. Coleman and K. F. Meyer (80) and W. A. Starin and G. M. Dack (81) have answered this negatively. They have concluded that the danger of botulism from toxin-free spores could follow only the ingestion of an enormous number.

Since the spores of *Cl. botulinum* are present in the soil and widespread in nature, they are undoubtedly swallowed in small numbers in dust and dirt and in uncooked fresh fruits and vegetables. Experience has shown that botulism does not occur in this way. Furthermore, if small numbers of *Cl. botulinum* organisms should grow in the intestinal tract and elaborate minute amounts of toxin, we should expect to find small amounts of antitoxin in the serum of animals. This is apparently not the case. The conclusion, therefore, can be drawn that the toxin of *Cl. botulinum* must be ingested to produce botulism.

Toxin of Cl. botulinum.—The toxin of *Cl. botulinum* is a true exotoxin, which gives rise to characteristic symptoms when injected into animals. The specific types give rise to specific antitoxins in the serum of immunized animals. The one irregularity, as mentioned before, is the observation of Pfenninger (40) that an Australian strain from Dr. Seddon produces a toxin which is neutralized by Type C antitoxin, but the antitoxin prepared for the Australian strain does not neutralize the toxin of American strains of *Cl. botulinum*, Type C.

As indicated in the beginning of the chapter, many types of food have been involved in outbreaks of botulism. However, many of these foods are relatively poor mediums for good growth and toxin production. If they were good mediums, it is likely that spoilage would be more evident in them, and they would be discarded rather than eaten. Growth and toxin of *Cl. botulinum* has been produced in chemically defined mediums (82) but, in, general, toxin of low potency occurs under these conditions. Very potent Type A toxin has been produced in mediums containing either powdered milk or casein, glucose, and clarified corn-steep liquor (83). Type A *Cl. botulinum* toxin has been crystallized from filtrates of cultures using this medium.

The toxin of *Cl. botulinum*, Type A, was obtained in crystalline form by two different groups of workers at approximately the same time (84, 85). The toxin behaves like a globulin with an isoelectric point of pH 5.6 and a total nitrogen of 14.1–14.3 per cent. The toxin crystallized readily in 0.10–0.30 saturated ammonium sulphate at 4° C., forming small needle-like crystals. An LD_{50} for the 20-gm. white mouse contains 4.5×10^{-9} mg. of nitrogen, and there are about 32,000,000,000 intraperitoneal LD_{50} per gram of dry toxin (86). The molecular weight based on studies of diffusion, apparent specific volume, and viscosity performed on crystalline materials was found to be 1,130,000 (87). By electrophoretic sedimentation and diffusion characteristics of the crystalline protein made by another group of workers (88), the molecular weight was calculated to be 900,000.

The elemental and amino acid composition of crystalline *Cl. botulinum*, Type A, toxin has been determined (89). The chemical entities which were found are shown in the accompanying tabulation.

	Per Cent		Per Cent
Lysine	7.74	Asparagine	20.10
Histidine	1.03	Serine	4.36
Arginine	4.63	Threonine	8.49
Tyrosine	13.50	Cysteine	0.268
Phenylalanine	1.17	Half-cystine	0.534
Tryptophan	1.86	Methionine	1.06
Valine	5.29	Proline	2.60
Leucine	10.30	Glycine	1.38
Isoleucine	11.94	Alanine	3.92
Glutamic acid	15.57		

The authors were unable to explain the extreme toxicity of the protein on the basis of their analytical data.

Type B toxin has been purified (90) by methods involving acid precipitations and working with the toxin on the acid side of its isoelectric zone. The purified toxin was not crystalline and appeared to be a slightly colored, simple protein, soluble in water on the acid side of the isoelectric range and relatively insoluble on the alkaline side and within the isoelectric range. Slight additions of salt did not favor increased solubility of the purified toxin. The molecular size, estimated with a sintered glass-membrane diffusion apparatus, was found to be about 60,000, in contrast to the much higher figure for the crystalline Type A toxin (900,000–1,200,000). Serologically, chemically, and physically the purified B toxin differs from Type A crystalline toxin. Its toxicity per milligram of nitrogen is only slightly less than that of Type A, but on a molar basis it would appear to be ten times less potent. The guinea pig is about three times more susceptible to the toxin than is the white mouse when the toxin is injected intraperitoneally.

Toxoids have been prepared from botulinum toxins, Types A and B (91). When these toxoids were tested in animals (92), a marked difference was found in the antigenic response of mice to fluid and to alum-precipitated botulinum toxoid. One dose of fluid toxoid induced little immunity in mice, whereas good immunity was obtained with a single dose of alum-precipitated toxoid. Guinea pigs developed immunity following single-dose injections of fluid, as well as of alum-precipitated botulinum toxoid. Botulinum, Type A, toxoid has been prepared from crystalline toxin by the addition of formaldehyde (93). One preparation consisted of one component electrophoretically, immunized mice in a dose containing 0.01 γ of toxoid nitrogen, and was twenty-four hundred times more active antigenically than was crude toxoid on the basis of nitrogen content.

Fluid and alum-precipitated botulinum toxoids, Types A and B, have been used to immunize a man (94). Excellent results have been obtained, and the method is practical in protecting laboratory personnel working with these toxins against accidents whereby the toxin may gain entrance to the body.

In contrast to the heat resistance of the spores, the toxins are susceptible to heat. Thus the data on the heat resistance of the toxin of Type A are summarized by Meyer (4). At 80° C. it is destroyed within 30 seconds to 6 minutes, at 72° C. within 2–18 minutes, and at 65° C. within 10–85 minutes. The toxin of certain strains of Type B have been found to require 15 minutes at

80° C. for destruction, whereas the toxin of type C strains seems to require more heat, i.e., 30 minutes at 80° C. (Bengtson), 6–8 minutes at 80° C. (Schoenholz and Meyer), and 15 minutes at 80° C. (Seddon). From these data it can be seen that toxins are not destroyed when foods are simply warmed. Therefore, before eating, the food should be boiled and during boiling thoroughly mixed so that a temperature sufficient to destroy the toxin will be attained throughout.

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CHAPTER V

Staphylococcus Food Poisoning

STAPHYLOCOCCUS food poisoning, like botulism, is produced by a toxin formed in the food before ingestion. It is probably the most common of all food poisonings, although we have no knowledge of the number of cases occurring annually, since it is not reportable. The symptoms are different from those of botulism; for they appear rapidly and are of shorter duration. Recovery is usually rapid and complete. A physician may or may not be summoned, and the next day the individual usually carries on his routine duties. Most persons afflicted are satisfied with the diagnosis of food poisoning or the incorrect diagnosis of "ptomaine poisoning." Since this type of ailment involves most individuals at one time or another during life, no great attention is paid to it, and it is soon forgotten. When large groups of people are attacked at banquets, in encampments, or in public institutions, however, publicity is given the event, and action is taken to determine the cause of the episode.

Staphylococci are common organisms found in the throats of individuals, on their skin as a causative agent of pimples, boils, and carbuncles, and in great abundance in the postnasal drip of patients recovering from colds.

HISTORY

The question of how long staphylococcus food poisoning has afflicted men is conjectural. Although staphylococci were not recognized before 1880, when Pasteur (1) observed them in pus, and they were not properly classified until 1884 (2), these organisms have undoubtedly caused food poisoning for centuries.

A few examples of food-poisoning outbreaks taken from the literature will suffice to illustrate this point. In 1830 (3) the comment was made:

> . . . certain articles of food have been frequently observed on the Continent to acquire poisonous qualities of a peculiar kind, and in a way which chemists and physicians have not hitherto been able to explain satisfactorily. Among these articles the most frequent are a peculiar variety of sausage, and a particular kind of cheese used in Germany; but both in France and Germany bacon and ham have been also several times found to acquire poisonous qualities analogous to those which characterize the sausage-poison and cheese-poison. A very elaborate inquiry into an accident supposed to have arisen from spoiled ham has just been published by M. Ollivier in the Archives Générales de Médecine. His investigations set completely at rest the common notion that such accidents arise from the accidental impregnation of the meat with metallic poisons; but he has not succeeded in discovering the real cause.
>
> In the instance which gave rise to his investigation, the master of a family purchased a ham pye at a pastry-cook's in Paris; and the whole family ate the meat of the pye the same day, and the crust on the following day. Three hours after dinner the master of the house was seized with general uneasiness, followed by cold sweats, shivering, violent pain in the stomach, and frequent vomiting; then with burning thirst, extreme tenderness of the belly, so that the weight of the bed-clothes could scarcely be borne, profuse purging, and colic of extreme violence. His daughter, twenty-seven years of age, and a child nine years old were similarly attacked. A physician who was called to their assistance soon after they were taken ill, drew up a minute report of the symptoms in each of his patients, and declared that they had a violent inflammation of the stomach, which he was inclined to ascribe to natural verdigris, or the carbonate of copper having been communicated by the pastry-cook's moulds. In a few days all the three individuals recovered under an antiphlogistic treatment. About the same period several accidents of the like nature occurred among the customers of this pastry-cook; and in consequence a judicial investigation was ordered. The shop being properly inspected it was found that every operation was conducted with due attention to cleanliness.

Chemical tests made of the meat and the paste separately and of the vomitus and stools did not reveal a trace of the commonly recognized chemical poisons—arsenic, copper, antimony, or lead.

In 1862 a food-poisoning outbreak caused by rabbit pie was described by John Buckenham (4) as follows:

> On the 19th of August, a family of ten persons residing in this neighbourhood, having at dinner partaken of a rabbit-pie, were all shortly afterwards seized with diarrhoea and violent pains in the abdomen, which continued throughout the greater part of the night. Some astringent medicine having been

administered, considerable relief was obtained. On the following morning, however, the precise cause of their illness not being suspected, several members of the family again partook of the remnant of the pie, reproducing the symptoms of the previous day in an alarmingly aggravated form, vomiting and violent cramps in the lower extremities being superadded.

Having been hastily summoned, I found on my arrival one of the patients in a state of collapse, pulseless, and insensible, and bathed in a cold, clammy perspiration. The others were also suffering more or less severely in proportion to the quantity of the poisonous food each person had eaten. . . .

From inquiries instituted in the matter, it has been ascertained beyond doubt that these sudden and remarkable symptoms were attributable solely to the rabbit-pie, as each person who partook of it was similarly affected, and the symptoms recurred in every instance in those who again ate of it, whilst those who partook more sparingly or avoided it escaped. The rabbits, and other materials composing the pie, it was proved were perfectly fresh and good, but were probably rendered unwholesome, though not unpalatable, by the process of cooking, having been baked beneath an impervious crust, which prevented the evaporation of the generated steam. It was afterwards permitted to stand for two or three days during very hot weather, which caused it to undergo a state of fermentation, as was observed from its peculiar appearance in the entire absence of jelly. Had the food been consumed when hot and freshly baked, or the confined steam freely permitted to escape by raising the paste, or otherwise affording sufficient ventilation, it is probable that no injurious effects would have resulted; at least such appears to me the only satisfactory explanation to account for the above remarkable symptoms.

An outbreak of food poisoning caused by eating pork brawn (molded product containing pieces of pork in a gelatin matrix made by prolonged cooking) was reported by Edward Mackey (5) in 1873:

Cases similar to the following deserve record, because they have a medicolegal interest, and may be required for reference and precedent.

On January 4th last, at Hampton-in-Arden, sixteen persons, including men, women, and children, aged from five to seventy-eight years, were suddenly attacked with violent vomiting and purging, accompanied in most cases with general and severe muscular cramps, soreness of eyes, and sense of burning and constriction in the throat. . . . Some seemed to be in serious danger; but, under appropriate treatment, all were convalescent next day. It was found that all had eaten some "pork brawn," and had begun to suffer two or three hours afterwards. All who had eaten any suffered more or less. The brawn had been bought from one provision-dealer in the village, a respectable man who could give no reason for its bad effects. He had eaten a little of it (perhaps two ounces), and had thought it particularly good. Of eighteen pounds made, six had been sold; the rest he himself gave into the custody of the police.

No mineral poisons were found in samples of the brawn subjected to chemical examination. In concluding, the author states:

> I certified to the absence of mineral poison and to the probability of formation of acrid fatty acids—a suggestion previously made by Dr. Wade.
>
> It may be observed in conclusion, that the dealer will never attempt brawn-making again, "for he suffered so much in his mind." His customers were considerate towards him, and no legal proceedings arose. Somewhat similar cases might not always end with as little mischief.

In 1879 Henry Leffmann (6), lecturer in toxicology in the summer school of Jefferson Medical College, called attention to the frequency with which cream puffs had been implicated in outbreaks of food poisoning. He writes: "I have made chemical examinations of portions of cream puffs that have caused trouble, but have not found anything that could be assigned as the cause of the action."

Since 1880 numerous bacteria have been found to cause disease and, as will be pointed out, the incrimination of staphylococci in food poisoning dates from this era.

Professor Victor C. Vaughan (7) in 1884 made the following statement with reference to food poisoning caused by cheese:

> It is well known that cases of severe illness follow the eating of some cheese. Indeed, such instances have been observed in various parts of the world for the past three hundred years. They are of frequent occurrence in the North German countries and in the United States. In England they are less frequently observed, while in France, where much cheese is used, we find no record of any such cases. In northern Ohio, a few years ago, the reputation of a large cheese factory is said to have been destroyed by the great number of cases of alarming illness occurring among those who ate of its product. In Michigan, during the past six months, nearly three hundred cases of cheese-poisoning have been reported.

Dr. Vaughan observed that the old, foul-smelling cheese, such as Limburger and Schweitzer, are alkaline in reaction and that poisoning never results from their use.

Professor Vaughan performed the following experiments with samples of poisonous cheese:

> I covered some bits of the cheese with 90 per cent. alcohol, agitated thoroughly, and filtered. The clear alcoholic filtrate, on being evaporated at a low temperature, left a large residue of fatty acids with possibly some contained nitrogenous bases. Having ascertained that nothing could be learned by feeding the cheese to the lower animals, I determined to experiment upon myself. I ate

> a piece of this alcoholic extract, about the size of a hazel-nut. It had a bitter, acrid taste, and in ten or fifteen minutes there were marked constriction and dryness of the fauces, much the same as that experienced while under the effect of atropia. Later there was considerable nausea, and I could readily believe that a larger amount would have caused vomiting. This experiment was repeated a number of times with the same result, and from it I conclude that the poisonous material, whatever it may be, is contained in the alcoholic extract. This would indicate a chemical poison and not a bacteric one. However, this chemical poison might be generated by the agency of bacteria. . . .
>
> Microscopical examination of the opalescent drops already mentioned, or of water which had been poured upon the cheese and allowed to stand for a short time, revealed bacterium termo and spherical bodies from ¾ to 1 micromillimeter in diameter, the latter having a rapid swinging motion. These spherical bodies are not the ova of small animals, for they are insoluble in ammonium hydrate. They may be micrococci, or the resting spores, of some bacillus.

Henry B. Baker, secretary of the Michigan State Board of Health, in 1884 sent some of the cheese which had caused illness to Dr. George M. Sternberg (8), surgeon, United States Army, who was stationed at Johns Hopkins University. Dr. Sternberg found that fluid from the cavities of the cut cheese, on direct examination, showed the presence of micrococci and no bacilli or other microörganisms. Cultures in broth when incubated at 38° C. for 24 hours contained only micrococci in pairs and in chains. Each of two rabbits inoculated subcutaneously with about 2 ml. of broth culture remained in good health. Rats and dogs fed the cheese developed no ill effects. A dog given 3 ml. of broth culture of the micrococcus and subsequently 10 ml. of an alcoholic extract of cheese remained well. However, Dr. Duggan, who assisted Dr. Sternberg with his experimental work, ate about 15 gm. of the cheese at 2:30 P.M., and at 5:00 P.M. he was attacked with nausea and vomiting and had one loose discharge from the bowels. He was not subject to similar attacks and ascribes the symptoms to the cheese which he had eaten. Dr. Sternberg concludes: "It seems not improbable that the poisonous principle is a ptomaine developed in the cheese as a result of the vital activity of the above mentioned micrococcus, or of some other microorganism which had preceded it, and had perhaps been killed by its own poisonous products."

It is of interest that Dr. Sternberg in his book entitled *A Text-Book of Bacteriology* (9), published in 1901, in chapter v, "Ptomaines and Toxalbumins," discusses the problem of cheese poisoning under a paragraph headed "Tyrotoxicon," as follows:

> First obtained by Vaughan in poisonous cheese, and subsequently by the same chemist and others in poisonous milk and ice cream. Chemically tyrotoxicon is very unstable. It is decomposed when heated with water to 90° C. It is insoluble in ether. From sixteen kilogrammes of poisonous cheese Vaughan obtained 0.5 gramme of the poison. The symptoms produced in man by eating cheese or milk containing tyrotoxicon are vertigo, nausea, vomiting, and severe rigors, with pain in the bowels attended with purging, numbness and a pricking sensation in the limbs, and great prostration.

In the years following the reports by Vaughan and Sternberg, micrococci were found repeatedly in large numbers in foods incriminated in food-poisoning outbreaks. Thus, Denys (10) in 1894 found them in beef; Owen (11) in 1906 recovered them from dried beef; and Barber's (12) classic work incriminated them in food poisoning due to milk.

A commission (10) reported the work of Dr. Denys, who was a professor at the University of Louvain. According to him, all but one child of a family ate meat from a cow dead of "vitullary fever," and, while the child remained well, all the others became sick, and one member died of an illness resembling cholera. Bacteriological examination led him to conclude that the trouble was due to the presence of pyogenic staphylococci. Furthermore, he ascertained that cultures of the organism exerted little or no effect when injected into the pleura of rabbits, and the commission itself ventured to state that the route of administration may be important. Owen (11), in 1906, corroborated the findings of Denys by reporting the acute intestinal intoxication of nineteen people within a few days in Kalamazoo, Michigan. The symptoms, characteristic of staphylococcus food poisoning, were enumerated in detail, and the board of health incriminated some dried beef, which seemed from subsequent work to have become contaminated only after it reached the particular store from which it was sold. It seemed normal in odor and in appearance, except for a slight greenish tinge in a few places; and detailed work by Dr. Novy indicated that cultures from this meat produced little or no effect when fed to various laboratory animals.

This subject was reopened in 1914 by Barber (12). Suffice it to say that he found that milk drawn from one-quarter of the udder of a cow which had been the seat of an attack of mastitis three years previously contained a white staphylococcus which was responsible for sporadic cases of acute gastrointestinal upsets on a certain farm in the Philippine Islands during the years 1909–13. This organism developed in sufficient numbers to be serious only in unrefrigerated milk or cream. It was small, tended to form diplococci, and liberated a toxic substance which was responsible for the upsets. No agglutinins were formed in the serum of those affected, and, as before, animals, including monkeys, exhibited no symptoms upon ingesting large quantities of milk cultures. The findings given in this excellent report remained unappreciated for fifteen years. The significance of this work incriminating staphylococci was not recognized, and food poisoning was with few exceptions ascribed to other bacterial agents. For example, in World War I two thousand soldiers in the German Army suffered an illness characterized by vomiting, diarrhea, and exhaustion, with symptoms appearing within 2–3 hours after eating sausages (13). The sausage was subsequently shown to contain cocci in large numbers and *Proteus vulgaris*. The investigator assigned the causative role to *P. vulgaris*.

In 1929 the role of staphylococci in food poisoning was rediscovered when Dr. W. E. Cary (14) brought to the laboratory two Christmas cakes which had been submitted to him by two of his physician-friends who knew he was interested in food poisoning. The cakes were three-layer sponge cakes with a thick cream filling, ornately iced and decorated with chopped pistachio nuts and maraschino cherries. They had been baked on December 23 or 24 in a commercial Italian bakery in Chicago and were delivered on the afternoon of December 24. They were presumably refrigerated at the bakery but were not kept in the icebox by the recipient. One cake had been served at various times on December 26 to five adults and three children and the other for dinner, also on December 26, to three adults. All eleven individuals who ate the cakes became ill. The victims were from four different families, so that other items of food could easily be eliminated. More than half of each cake was available for laboratory study, and laboratory work was begun 15 hours after the cakes were first cut.

Four monkeys were fed a saline suspension of the whole cake by stomach tube, and three mice received a saline suspension of the cake substance orally. None showed ill effects. Since the heavy metals, mercury and arsenic, were not found in the cake and no *Salmonella* organisms were isolated when appropriate cultures were made, the most logical alternative left was to test the material on human volunteers. It was thereafter found that, of the various components of the cake, the sponge cake itself, after removal of the frosting and filler, was responsible for the trouble.

A bacteriological examination of the cake substance revealed nineteen different types of colonies. Three of these were further studied, as they were predominant. A filtrate from a veal-infusion broth culture of only one of these, incubated for 40 hours, caused a rabbit when injected intravenously with 4 ml. of the filtrate to develop a profuse watery diarrhea, to lose 170 gm. of its body-weight (7 per cent) in 5 hours, and to die 12 hours later. In addition, 3 human volunteers drank, respectively, 25 ml., 10 ml., and 5 ml. of the filtrate which was lethal to the rabbit. Typical symptoms, beginning after 3 hours, occurred in the person drinking the 25-ml. portion. The volunteers who took the smaller amounts of filtrate (10 ml. and 5 ml.) experienced minor sensations of nausea, chilliness, and gastrointestinal distress after 3 hours. The organism from which the toxic filtrate was prepared was a yellow hemolytic staphylococcus and, in smears from broth cultures, was arranged in pairs or chains rather than in clusters like many of the staphylococci. In this respect the morphology agreed with Barber's (12) description.

The proof of the causative role of staphylococci in food poisoning has rested upon experiments with human volunteers, since none of the ordinary laboratory animals are susceptible by feeding enterotoxin in amounts which affect man. The long delay in recognition of the role of this organism in food poisoning may be attributed chiefly to the immense amount of publicity given *Salmonella* or paratyphoid organisms in food poisoning. In fact, when in earlier outbreaks *Salmonella* was not found, various means (discussed under *Salmonella*) were devised to detect their heat-stable products, since it was falsely assumed that they gave rise to food-poisoning symptoms in man. Paradoxically, perhaps, knowledge of the ubiquity of the staphylococcus has contributed to the delay in the recognition of its importance in food poisoning.

The "modern era" of staphylococcus food poisoning, therefore, dates from 1930. Since that time our knowledge of the subject has increased rapidly, and this most common of all types of food poisoning is now recognized throughout the civilized world.

EPIDEMIOLOGY

The conditions necessary for an outbreak of staphylococcus food poisoning are as follows: contamination of a food with enterotoxin-producing staphylococci, a suitable food in which the organisms can grow, and the keeping of this food for a sufficient time at a temperature compatible with growth.

Many different types of foods have been involved in this food poisoning. Those previously referred to include ham pie, rabbit pie, pork brawn, cream puffs, cheese, beef, dried beef, milk, sausage, and cream-filled cake. F. C. Kelly and G. M. Dack (15) list additional outbreaks due to cheese, cream-filled cake, chicken gravy, cream-filled éclairs and tarts, custard-filled doughnuts, chocolate éclairs, milk, and tongue sandwiches. Many other foods have been incriminated, such as hams or tongue (16), hollandaise sauce (17), *kamaboko* (fish sausage) (18), liver sausage (19), goat's milk (20), pressed pickled beef (21), ice cream (22), chicken salad (23), pineapple trifle (24), and bread pudding (25).

A. W. Fuchs (26) studied outbreaks from water, milk, and other foods in 1939. Large numbers were reported by a few states and a small number or none from others. The reporting of these outbreaks was out of all proportion to the relative populations of the states. Thus Fuchs states:

> For example, 1 state, with only one-tenth of the country's population, reported practically one-half of all the water-borne outbreaks. Again, 3 states reported over one-half of all the milk-borne outbreaks. Similarly, two states accounted for more than half of all the food-borne outbreaks. From our knowledge of the quality and extent of the public health activities of these states it would be unreasonable to assume that they are below average in environmental

sanitation. On the contrary, the logical explanation probably lies in their efficient epidemiological organization for uncovering outbreaks and in their willingness to report such outbreaks [26].

Fuchs points out that, for the year 1939, outbreaks of food poisoning traced to pies and pastry were the most numerous, and those due to pork and pork products held second place. He does not state how many in this group are due to staphylococci or to other causes. In upstate New York from 1935 to 1939, 17 outbreaks (27) of staphylococcus food poisoning were reported that were due to cream-filled bakery goods. One of these involved 700 individuals, and the 17 outbreaks gave rise to 1,246 cases.

Staphylococcus food poisoning is widespread over the civilized world. In England most outbreaks of food poisoning reported have been alleged to be due to *Salmonella*. However, G. S. Wilson (28) states:

> There is no exact information on the frequency of food poisoning in this country. The disease is not notifiable, cases are often sporadic, the true nature may not be recognized, and the illness may be so transient that no doctor is called in. Available evidence suggests that the disease is common, but that the great majority of sporadic cases and many of the smaller outbreaks never come to the notice of the health authorities. Before the war, Scott estimated that about one-third of all cases of food poisoning were of the staphylococcal toxin type.

Fish sausage (18) is referred to by the Japanese. Timmerman (19) in Holland describes an outbreak due to liver sausage. He tested a filtrate, from the staphylococcus involved, on a human volunteer, who developed symptoms of acute food poisoning. He is of the opinion that a larger number of cases of food poisoning are caused by staphylococci than is generally believed and that poisonings that develop after the eating of cheese are often caused by these organisms. Timmerman discounts the role of *Bacterium coli* in cheese poisoning, since *Bact. coli* is present in almost 60 per cent of samples of cheese and causes no poisoning.

The growth requirements of the staphylococci are easily fulfilled in many different types of food, as is evidenced by the many different foods which have been reported. Custard-filled bakery goods are probably responsible for the greatest number of outbreaks, while ham or tongue ranks next. Slocum and Linden (16) found that the consumption of "ready-to-eat" hams or tongue was responsible for 20 outbreaks of food poisoning, apparently due to staphylococci, in which at least 1,028 individuals were affected. V. C. Tipton and D. F. Smiley (29) reported 3,884 cases in 6 outbreaks occurring in the Navy, all of which were traced to ham.

In recent years bacteriophages have been used to identify strains involved in food-poisoning outbreaks and to detect similar strains in food handlers. By this method it has at times been possible to identify tentatively the source of contamination (22, 30, 31). Occasionally, repeated outbreaks have been traced to the same food handler (22). However, the establishment of phage types limited to food-poisoning strains is not yet on a firm basis.

SYMPTOMS

The symptoms of staphylococcus food poisoning appear in about 3 hours, occasionally from 1 to 6 hours, after ingesting food containing the enterotoxin. The incubation period is influenced by the amount of enterotoxin consumed and the susceptibility of the individual. Usually, the first symptom observed is salivation, which is subsequently followed by nausea, vomiting, retching, abdominal cramping of varying severity, and diarrhea. In severe cases blood and mucus may be observed in the stools and vomitus. In mild cases there may be nausea and vomiting without diarrhea, or there may be cramping and diarrhea without vomiting. Headache, muscular cramping, and sweating often occur if symptoms are moderately severe. Marked prostration accompanies the vomiting and diarrhea in severe cases. In such cases fever may occur, or the temperature may be subnormal. G. A. Denison reported one case (32) in which the temperature dropped from 98.6° to 96.0° F. and returned to normal after 9 hours. The blood pressure in this same case dropped from 120/80 to 60/40. Staphylococcus food poisoning in otherwise normal individuals is usually not fatal. A few fatal cases have occurred in which inadequate treatment was given. For example Denys (10) reports the death of one member of a family made ill with contaminated beef, the illness resembling cholera. In another outbreak two children (20), three and four years old, died within 24 hours after each drank 250 cc. of milk of a goat suffering from acute suppurative mastitis due to *S. aureus*. Recovery is usually rapid in from 1 to 3 days. Some patients, however, may not completely recover for a week or more. Individuals may vary considerably in their susceptibility to the enterotoxin. One human volunteer (33) vomited following the ingestion of 0.5 ml. of the filtrate of a staphylococcus culture and was nauseated on one occasion by only 0.25 ml. The same filtrate was fed in increasing doses up to 13 ml. to another volunteer (33) without causing active symptoms. In another experiment (15) involving a sandwich contaminated with staphylococci, one individual became severely ill, although another was not affected. Therefore, since susceptibility among different individuals varies, the size of the dose of the enterotoxin is not the only factor determining the severity of symptoms. Similar data from reported attacks have been assembled in Table 9. The data are fairly reliable, since single families were for the most part involved and the incriminated food was uniform.

TABLE 9

SUSCEPTIBILITY OF INDIVIDUALS TO STAPHYLOCOCCUS FOOD POISONING

Outbreak		No. of People Eating Incriminated Food	No. Ill	Per Cent Ill
1 (32)		11	11	100
2 (34)		325	....	60–70
3 (35)		3	3	100
4 (36)	Family P	4	4	100
	Family W	3	3	100
	Family B	4	4	100
	Family B (No. 2)	4	3	75
	Family O	6	6	100
5 (37)	Family M	2	2	100
	L luncheon	5 or 6	5	82–100
	Family T	2	2	100
	Family P	4	4	100
	Family McQ	5	5	100
6 (38)		102	96	94.1

Since some individuals do not develop symptoms of food poisoning when they ingest staphylococcus enterotoxin, the question arises as to the relative susceptibility of the population.

There are, unfortunately, many sources of error involved in obtaining such information when large numbers of people are involved. Thus the food involved may not contain enterotoxin at the time of distribution but may develop it later under suitable conditions of time and temperature. It may be kept by one housewife for a time and at a temperature different from that of another. Accordingly, a single outbreak involving the same item of food from a central source will not contain a standard amount of enterotoxin from which to judge the relative susceptibility of individuals in a large outbreak. Another source of error is the difficulty of obtaining accurate data from a large number of individuals. It is sometimes difficult for one investigator to see all the persons involved. Also, the amount of certain items of food eaten varies with the individual, so that the dosage may not be uniform even in a family group. Finally, outbreaks in which only one or two in a family are affected are probably never reported. From these data it is evident, however, that when enterotoxin is ingested in food, few escape illness.

TREATMENT

In staphylococcus food poisoning, vomiting and diarrhea are generally severe, and it is unnecessary to empty the stomach with a stomach pump or give cathartics to free the rest of the gastrointestinal tract of enterotoxin. In severe cases prostration is acute, and blood pressures of 60/20 have been observed. These symptoms of shock are due to the loss of body fluids, resulting in dehydration and disturbances of salt balance, and should be counterbalanced by giving normal saline solutions parenterally. The amount of fluid given should be governed by the age of the patient and the severity of the vomiting and diarrhea. No specific drug or serum therapy is of value.

LABORATORY DIAGNOSIS OF OUTBREAKS OF STAPHYLOCOCCUS FOOD POISONING

One of the most characteristic features of staphylococcus food poisoning is the shortness of the time interval between eating the incriminated food and the onset of symptoms. This incubation period may vary from 1 to 6 hours but is usually about 2½–3 hours. If an outbreak of food poisoning occurs within this incubation period, it is helpful to the laboratory worker to have the epidemiological evidence regarding the suspected item or items of food. By submitting a list of foods served at the meal in question, together with the number of individuals ill and the number not ill, specific items of food involved can often be determined. Much time can thereby be saved in searching for the causative agent in the specific food or foods common to those who were ill.

Weighed samples of solid food and measured samples of liquid food are used to ascertain what bacteria are present. The solid food is ground in a sterile mortar with sterile sand and saline. Decimal dilutions of the food samples are made and plated in veal-infusion agar. The plates are counted at the end of 24 and 48 hours. Blood agar plates and selective mediums (eosin–methylene blue or *Salmonella-Shigella* agar) are streaked to rule out gram-negative intestinal pathogenic organisms, such as *Shigella* and *Salmonella* types.

In the case of staphylococcus food poisoning, the involved food usually contains hundreds of millions of staphylococci per gram. It is theoretically possible, although the author has never encountered it, that staphylococcus food poisoning may occur from food in which the organisms, after growing and producing enterotoxin, are destroyed by heating prior to serving. Such a condition would be extremely difficult to prove and would necessitate producing appropriate symptoms in a human volunteer by feeding the food in question while eliminating other possible poisoning by chemical or other tests. In this connection one outbreak is reported which involved cooked as well as uncooked food. In this outbreak, out of 160 persons (18) who ate *kamaboko* (fish sausage), 110 were poisoned, including 16 who thoroughly heated the sausage. The time and temperature of heating were not given, but apparently the food was sufficiently heated to destroy the staphylococci but not the enterotoxin. If the majority of people eating the food are affected, if hundreds of millions of staphylococci are present in it, and if the incubation period from the time of eating to the development of symptoms is 2½–3 hours, the outbreak is almost certainly due to staphylococcus food poisoning. Circumstantial evidence is, however, not usually so precise. For example, such a small amount of enterotoxin may be formed that it will be sufficient to affect only the most susceptible individuals. Staphylococci are ubiquitous, and millions are contained in many of our foods without causing illness, since many strains do not produce enterotoxin. On the other hand, food-poisoning strains do not produce potent enterotoxin in certain foods.

The ideal and conclusive test for identifying staphylococcus food poisoning would be to detect enterotoxin in the food directly, but this as yet has not been developed by chemical or other laboratory methods. Small amounts of toxin may be detected only by using the highly susceptible human volunteer. The next best step, if large numbers of staphylococci are found, is to determine whether they are able to produce enterotoxin under experimental conditions.

Staphylococci are grown in Kolle flasks containing 75 ml. of 1 per cent veal-infusion agar of pH 6.0 (39). The flasks are placed in vacuum desiccator jars, the jars are partially exhausted, and 20 per cent carbon dioxide is admitted. After an incubation of 48-72 hours at 37° C., the agar is removed from the flask and squeezed through four layers of gauze. The fluid portion is then centrifugalized at high speed to remove agar and most of the bacteria. It may then be filtered through a Berkefeld "N" or Seitz filter, or it may be fed directly to monkeys without filtration. A simplified medium for enterotoxin production has been prepared by G. O. Favorite and W. Hammon (40) (see accompanying table). The pH is adjusted to 7.6 with 40 per cent sodium hydroxide, and the volume of the medium is brought to 99 ml. It is then autoclaved for 10 minutes at 10 pounds pressure. When cool, 1 ml. (0.25 gm.) of sterile 25 per cent glucose is added. The medium is poured into hexagonal, narrow-necked Pyrex nursing bottles of 250-ml. capacity. After inoculation, the bottles are placed in a large ana-

	Per 100 Ml.
Casein hydrolysate	1.5 gm.
Thiamin chloride (vitamin B_1)	0.5 γ
Nicotinic acid	120 γ
Distilled water to a volume of	90 ml.
Phenol red (0.2 per cent)	0.4 ml.

erobic jar from which all but 5 per cent of air is removed and replaced by a carbon dioxide–oxygen mixture. As soon as the jar is opened, the cotton plugs of the bottles are quickly replaced with rubber stoppers. The bottles are slowly rotated during incubation. Enterotoxin is produced in this medium under these conditions in an amount equal to that produced in semisolid veal-infusion agar in an environment of 20 per cent carbon dioxide.

The test for enterotoxin is made by feeding whole cultures or filtrates or by injecting boiled cultures or filtrates into the abdominal cavity or blood stream of animals. The amount of enterotoxin in such mixtures has to be large if it is to be tested in animals because no experimental animal has been found that will react to the small amounts of enterotoxin sufficient to cause severe reactions in man.

The best test animal to feed or inject with potent enterotoxin appears to be the rhesus monkey. Thus E. O. Jordan and J. McBroom (41) found that *Macaca mulatta* reacts similarly to man when fed filtrates containing enterotoxin in amounts as small as 2–5 ml. and reacts more uniformly when larger doses are given. The usual test involves giving the monkey 50 ml. of culture or filtrate by stomach tube.

> Usually about an hour and a half after feeding, the animal becomes somewhat pale; increased salivation and swallowing movements are noted; the monkey may bend over in the corner of its cage with its forearms folded across its abdomen. The pallor and salivation increase; there is usually a short premonitory period marked by regurgitation. At about the beginning of the third hour vomiting sets in. This may be mild but is often profuse and projectile. Paroxysms of vomiting are apt to recur over a period of an hour or so. During this time the animal is very pallid and abject. Diarrhea may be a marked feature; in two cases in which large amounts of toxic material were fed, there was profuse diarrhea without vomiting; similar reactions have been noted in man. Recovery sets in rather abruptly after several hours, and on the following day the monkey may appear normal except for loss of weight. All of the animals that were fed recovered, although in a few instances diarrhea persisted several days [39].

Jordan and McBroom (41) also used the following species: the red spider (*Ateles geoffroyi*), black spider (*A. ater*), black howler (*Alouta palliata inconsonans*), and white-throated monkeys (*Cebus capucinus capucinus*). These animals exhibited definite symptoms of diarrhea, lack of appetite, and malaise, and, in one instance, vomitus was found on the floor of the cage. Subsequently, E. Davison, G. M. Dack, and W. E. Cary (42) found that vomiting and other typical symptoms regularly follow the intravenous injection of rhesus monkeys with boiled filtrates from cultures of food-poisoning staphyloccoci in doses of 1 ml. per kilogram of body-weight. Symptoms have also often been produced with as small an amount as 0.2 ml. per kilogram.

A disadvantage of using monkeys is that they are expensive to buy and to maintain. Another important objection is their resistance to the oral administration of enterotoxin as compared to the relative susceptibility of man. In addition, the monkey is not so easily handled as are many animals and is likely to become resistant to enterotoxin after several feedings. A source of error in these tests has been pointed out by F. C. Minett, who states: "Monkeys frequently regurgitate material into the mouth and then slowly swallow it, but it is doubtful if any significance should be attached to this act, since it was observed at times after unsown filtrate had been fed" (43). It may be that some of the discrepancies in the results reported by different investigators may be explained upon this basis.

The cat has also been used extensively in testing for staphylococcus enterotoxin. C. E. Dolman, R. J. Wilson, and W. H. Cockcroft stated: "The cat is more akin to human beings than are rodents in dietary and excretory habits; is less prone to vomit than the dog; and is a more convenient and economical experimental animal than the monkey"(44). These workers prepare filtrates by inoculating plates of semisolid nutrient agar medium with the strain of staphylococcus under investigation, harvesting after 40 hours' incubation at 37° C. in an atmosphere of 30 per cent carbon dioxide and 70 per cent oxygen. The suspension is freed from agar by passing through cheesecloth and is filtered through a Seitz filter. To the filtrate 0.3 per cent solution of formaldehyde (U.S.P.) is added, and the formalinized filtrate is kept in the incubator at 37° C. until rabbit- and sheep-cell hemolysins (alpha [α] and beta [β] toxins) are no longer detectable. It is then ready to test on kittens for the presence of enterotoxin. The following statement is made:

> In our more recent experiments, advantage has been taken of the greater heat stability of the enterotoxin as compared with the alpha and beta toxins. Filtrates have been placed in a boiling water bath for ½ hour prior to injection. Only negligible amounts of alpha and beta toxins survive this treatment, but enterotoxin, where present, proves heat-stable [42].

These workers found that as little as 0.5 ml. of a potent filtrate injected intraperitoneally will cause a severe reaction in a 6–8-week-old kitten weighing 350–550 gm., while 3 ml. of a filtrate from innocuous strains, or of formalinized broth, will cause no upset. They found that adult cats may be used for the test but are harder to handle and seem relatively less sensitive than kittens.

M. J. Surgalla and K. E. Hite (45) made a comparative study of the production of enterotoxin and of alpha and beta hemolysins by a number of strains of staphylococci. They found that all the strains which were enterotoxic when fed to monkeys also produced alpha lysin, whereas only some of the cultures produced beta lysin. They concluded that staphylococcal enterotoxin and hemolysins are separable and distinct entities.

G. J. Kupchik (46) essentially substantiated this work, but others have found that uninoculated medium alone produces symptoms in kittens (47). The fact that the intraperitoneal injection of sterile broth into kittens may give rise to food-poisoning symptoms is sufficient warning that each test should be controlled by injecting the test kitten with sterile broth first to see whether a reaction is elicited. If no food-poisoning symptoms follow, then unknown boiled cultures or filtrates prepared from the same medium may be tested. The reaction following the injection of kittens with sterile broth may or may not be due to peritoneal irritation. Certainly, our bacteriological media contain a complex mixture of chemicals. No two brands of peptone are alike, and no two lots of the same brand are identical chemically. The reactions occurring with sterile broth may well be due to the toxicity of the medium itself. Whether the simplified medium recommended by Favorite and Hammon will prove practicable and obviate this difficulty is problematical.

Davison, Dack, and Cary (42) found that the assay of enterotoxic substances by the intravenous injection of kittens with specimens to be tested offers certain advantages over other methods. Since, as in monkeys, it is a more delicate test, only a small inoculum is required, and susceptible animals may be used repeatedly. W. Hammon is enthusiastic about this test and says:

> It would appear from the results of this study that the intravenous cat test for enterotoxin is one which can be readily applied to heat treated culture filtrates in any health department laboratory. Since adult cats or large kittens can be used, no difficulty should be encountered in acquiring and keeping the necessary animals, and each may be used three or four times before developing too great a tolerance. Negative results, however, should be confirmed by inoculation of at least two previously unused animals. A healthy cat, given a meal shortly before inoculation, should react by actually vomiting before any test is considered positive. This method of inoculation has definite advantages over the intra-abdominal route [48].

It must, however, be used on a large scale in many laboratories before its real merits may be assessed. The introduction into the blood stream of various protein degradation products, such as are found in the ordinary laboratory media, may give rise to false positive tests, as in the case of the intraperitoneal kitten test.

Other animals which have also been used to test staphylococcus enterotoxin include frogs, white mice, wild mice, white rats, guinea pigs, rabbits, puppies, dogs, and canaries. Many of these species do not have a vomiting reflex, and diarrhea has been used as a criterion of reaction. At the November, 1941, meeting of the Society of Illinois Bacteriologists, Drs. E. W. Hopkins and E. F. Poland reported that young pigs are good test animals. They injected boiled staphylococcus filtrates intraperitoneally into young pigs and observed retching in a large majority of the animals. They also found that sensitivity to enterotoxin was retained after two or more injections.

CONTROL OF STAPHYLOCOCCUS FOOD POISONING

One of the most important problems of the public health worker is the control of staphylococcus food poisoning. Staphylococci may be found in abundance in nature. They are carried in the throat and are found in great numbers in the postnasal discharge following common colds. They cause pimples and carbuncles and other infections in man and may be isolated from the air of rooms. Fortunately, not all strains produce enterotoxin, and not all foods are favorable media. Nevertheless, it is obvious that foods exposed to the air may become contaminated with this coccus. Therefore, since some foods serve as excellent nutrient media for staphylococci, conditions should be made unfavorable for the production of enterotoxin. This may be accomplished simply by refrigeration, since M. Segalove and G. M. Dack (49) have shown that enterotoxin is not formed in periods up to 4 weeks at a temperature of 4°–6.7° C., even though other conditions necessary for enterotoxin production are fulfilled.

One of the great practical difficulties is the education of the public. If a food does not smell or taste spoiled, the layman thinks it is safe for eating. The reverse is, unfortunately, often true. In the case of staphylococcus food poisoning, the product may contain sufficient enterotoxin to produce violent illness and yet have no odor of spoilage or abnormal taste. One group of foods which has given rise to many outbreaks of food poisoning is custard-filled bakery goods. J. Stritar, G. M. Dack, and F. G. Jungewaelter (50) worked out a simple method of reheating custard-filled puffs (pastry shells filled with custard) and éclairs (small oblong cakes filled with flavored custard and glazed over or frosted) for a period of time and at a temperature sufficient to kill staphylococci without impairing the flavor or appearance of the product. At a temperature of 109.6° C. (375° F.), for 30 minutes, the puff or éclair is not damaged, even though exposed directly to this heat in the oven. The baker needs only to return filled shells to the oven in an ordinary pan. At higher temperatures excessive browning occurs unless egg whites are substituted for milk in the manufacture of the shell. A recommendation for routine use would be 30 minutes at temperatures ranging from 190.6° C. (375° F.) to 218.3° C. (425° F.). This principle of heating foods is old, and its public health significance has been well established, as, for example, in the pasteurization of milk. F. A. Korff (51) reported that about fifteen bakeries in Baltimore were reheating custard-filled pastries in 1936 and finding it satisfactory. F. W. Gilcreas and M. B. Coleman (52) recommended reheating custard filling in éclair shells for 15 minutes at a temperature of 216°–220° C. (420°–428° F.). They reported that rebaking éclairs, chocolate cream pie, and Boston cream pie for 20 minutes at 216° C. did not impair the appearance or palatability of the pastries. They concluded as follows: "The adoption of this procedure in bakeries should reduce greatly the incidence of food poisoning induced by the enterotoxins of staphylococci in custard-filled products." Food poisoning from bakery goods is still prevalent. Unfortunately, many individuals do not change their long-established methods until they suffer themselves.

Since some bakeries have encountered more than one outbreak of staphylococcus food poisoning from their products, an attempt has been made in certain laboratories to look for carriers of food-poisoning staphylococci among bakery employees. Wilson (53) conducted laboratory investigations over a period of a year on the personnel of one bakery. In an outbreak occurring in March, 1938, he found that a strain of staphylococcus isolated from toxic pastry was the same as that found in the nose and throat of the owner of the bakery incriminated. This man harbored the same strain from March, 1938, to April, 1939, in spite of medical treatment. Wilson states:

> This person had apparently conveyed infection to other members of the bakery staff and to the utensils and food; and the same strain of staphylococcus was isolated from the cream-filled pastry on both occasions. It is of further interest to note that the persons implicated in each outbreak showed no visible signs of infection. This fact is rather disturbing when one considers the control of this type of food poisoning. Figures quoted by several authors show that the incidence of staphylococci in the noses of the general population is high. The proportion of these strains that may produce food poisoning is not known with certainty, but several surveys of strains from various sources indicate that roughly 40 per cent of these are enterotoxigenic, as shown by the kitten test. If these observations be true, the probability of food contamination, with possible food intoxication, is very great, since the nose and throat microflora provide a vast well of potential infection.

The method of correlating phage-susceptible strains of staphylococci (22, 30, 31) in food implicated in outbreaks with those isolated from food handlers may aid health officers in locating the source of some staphylococcus food-poisoning outbreaks and preventing recurrent outbreaks from the same source. It is possible, with the great abundance of staphylococci in nature, that a person may not carry an enterotoxic strain one day and may carry it the next day. In other words, there is always a potential danger of this type of food poisoning. On the other hand, workers with sinus infections or recurrent boils or colds probably should be excluded from the handling of food products.

The rapidly cured hams that are now on the market have given rise to many outbreaks of food poisoning. Many of the packers and the Bureau of Animal Industry are well aware of the problem. The process involved in the heating of the hams during their manufacture is designed to be effective against *Trichinella* as well as staphylococci, so that when hams leave the packer they are safe. But they may easily become contaminated, and the process of standing in a warm environment for several hours, as in a kitchen, may be favorable for the growth of staphylococci and the production of enterotoxin. The important procedure in the control of food poisoning from cured-meat products is to educate the public and the retailers to the fact that products such as ham are perishable and must be kept under refrigeration. It is a well-established fact that staphylococci grow very well in ham without producing signs of spoilage as evidenced by an abnormal odor or taste.

With the increased consumption of frozen foods, attention has been given to the potential hazard of staphylococcus food poisoning in these products, of which there is a great variety. The food-poisoning staphylococci have been found to grow in low-acid foods (54), such as peas, corn, salmon, and shrimp. In canned string beans, spinach, and asparagus less abundant growth occurred. In test-tube preparations of canned roast beef (55), corned beef, and potted meat, luxuriant growth occurs, and the organisms survive for at least 60 days at 22° and 37° C. when loss of moisture is prevented. Foods (56) such as chicken à la king, creamed tuna, ham à la king, creamed salmon, turkey and noodles, and cooked shrimp were found to be excellent mediums for the growth of food-poisoning staphylococci. Jones and Lockheed (47) found that, of 50 strains of micrococci isolated from frozen vegetables, 12 were able to produce enterotoxin. Several outbreaks (22, 57, 58) have been traced to ice cream. One outbreak has been reported from frozen fish (59).

Food-poisoning staphylococci have been found to survive freezing in creamed food products (56), such as chicken à la king, ham à la king, creamed tuna, and creamed salmon, when experimentally inoculated into these products and stored at −18° C. for 9 months. In a project at the Hormel Institute (60) supported by the Navy, microörganisms were suspended in nutrient broth, milk, and liver-agar paste in 1.5 ml. of suspension containing 1×10^4 to 1×10^5 organisms per milliliter and sealed in Pyrex test tubes, 10 × 75 mm. A rapid freeze in acetone dry-ice bath (−60° to −70° C.), an intermediate freeze in solid carbon dioxide icebox (−55° C.) and a slower freeze in a deep-freeze unit with a temperature of approximately −22° C. were carried out. The freezing times were 1, 30–60, and 150 minutes, respectively. Samples were thawed in a 35° C. water bath. The statement is made that the gram-positive coccus, *S. aureus*, "appeared to be practically unaffected by any method of freezing or by any type of suspending medium." From these experimental data it is evident that viable food-poisoning staphylococci are not destroyed by freezing. Since staphylococci do not produce enterotoxin at temperatures of 4–6.7° C. after a period of 4 weeks (49), it is obvious that only after incubation at higher temperatures is there a danger of food poisoning.

In summary, it is evident that many of the kinds of foods which are frozen provide excellent nutritional conditions for the growth and enterotoxin production of food-poisoning staphylococci. Frozen foods are subject, as are all foods exposed to the atmosphere, to contamination with these microörganisms. If errors in processing occur, so that enterotoxin is formed in the food before freezing, a potential danger from staphylococcal food poisoning exists. Furthermore, if mechanical failure of refrigeration should occur during storage, which would permit the formation of enterotoxin, the stage would be set for an outbreak of this poisoning.

In summarizing the control of staphylococcus food poisoning, several factors must be kept in mind. First, there must be sufficient contamination of the food with an enterotoxin-producing strain of staphylococcus. Second, the food must be a good medium for the growth of the organisms and the production of enterotoxin. Third, the food must remain at about room temperature or above for several hours. Of all these factors, temperature is the most practical to control.

ENTEROTOXIN-PRODUCING STAPHYLOCOCCI

Some strains of staphylococci have been repeatedly tested for enterotoxin on human volunteers over a period of years and have never been found to produce it, even though they may produce powerful hemotoxins and lethal and dermo-necrotic toxins.* This has been the experience not only of the author but also of C. E. Dolman (61).

The percentage of the total number of strains of staphylococi which are capable of producing enterotoxin is not known because, to date, no satisfactory test has been developed for differentiating the enterotoxin- from the nonenterotoxin-producing strains. Some years ago Dolman (61) worked with strains of staphylococci which produced potent hemolysins, dermotoxins, and lethal toxins but did not produce illness in 42 volunteers on 110 occasions. A strain which he received from Dr. E. O. Jordan, however, yielded a filtrate of which 2 ml. caused severe gastrointestinal disturbance in 3–9 volunteers and a lesser degree of disturbance in 4 others. On the basis of his experiments he felt that the number of strains of staphylococci which produce enterotoxin is small as compared to the total number.

R. V. Stone (62) developed a cultural method for classifying staphylococci of the food-poisoning type by testing cultures in a special gelatin medium for gelatin liquefaction. Any degree of liquefaction was a positive test and indicated an enterotoxin-producing strain of staphylococcus, whereas no liquefaction indicated a negative strain. The test cultures were incubated exactly 24 hours at 37.5° C. In using Stone's medium, B. D. Chinn (63) found that staphylococci incriminated in food poisoning cannot be differentiated by gelatin liquefaction. G. H. Chapman, C. W. Lieb, and L. G. Curcio (64) found that the Stone reaction was positive in 70.5 per cent of typical food-poisoning staphylococci and positive in 27.6 per cent of strains with similar properties but isolated from nonfood-poisoning sources. Kupchik (46) investigated the reaction to Stone's gelatin medium of forty-four strains of staphylococci, many of which produced enterotoxin. He found that Stone's medium failed to exhibit any differential value. It is evident that the gelatin-liquefaction test developed by Stone is not an absolute criterion of enterotoxin production.

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* Hemotoxins as tested on rabbit and sheep red blood corpuscles; lethal toxins as tested by the intravenous injection of mice with filtrates; dermo-necrotic toxins as tested by the intradermal injection of rabbits with dilutions of filtrate.

J. Stritar and E. O. Jordan (65) studied ninety-four strains of staphylococci from various sources. They found that the food-poisoning strains do not constitute a clearly marked group. Neither in biochemical, in hemolytic, nor in agglutinative characters is there evidence of homogeneity. They concluded that "the power to provoke food poisoning is not limited to any recognizable variety of staphylococcus."

Staphylococci grow well in cured meats. They grow in salt meat, which prohibits the increase of spore-forming and non-spore-forming rods, and in chicken containing 5 and 10 per cent of salt (15). They are found in great numbers in wrapped minced ham and mayonnaise sandwiches purchased at drug stores (15). Hucker (66) has shown that staphylococci will grow in concentrations of salt and sugar that are inhibitory to many bacteria. The organism is, therefore, not affected by certain reagents, such as salt and sugar, which are used in partially preserving certain foods. As a matter of fact, the addition of suitable concentrations of salt may serve to prevent the growth or to kill certain spoilage bacteria which might compete for growth with the staphylococcus.

STAPHYLOCOCCUS ENTEROTOXIN

Production of enterotoxin in synthetic mediums.—M. J. Surgalla (67) studied the production of staphylococcal enterotoxin in synthetic mediums consisting of amino acids, vitamins, inorganic salts, and glucose. He was able to detect enterotoxin in the supernatants of cultures grown in mediums containing from 2 to 16 amino acids as the source of organic nitrogen. The amino acids arginine and cystine were present in the most simple medium. Evidence was not obtained that amino acids unessential for growth were necessary for enterotoxin production. Both growth and toxin production were reduced in the more simple mediums, which Surgalla attributed to the amount of available nitrogen in the mediums. Growth did not take place in the absence of glucose in a medium containing only three amino acids. In mediums containing glucose in concentrations ranging from 0.2 to 20.0 per cent, enterotoxin was demonstrated. Less enterotoxin was produced in the simple, as compared to the more complex, mediums.

Time and temperature for the production of enterotoxin.—It is important to know the time and temperature necessary for staphylococci to produce sufficient enterotoxin in foods to give rise to food poisoning in man. The answer to this question may be obtained by a survey of the literature. The production of enterotoxin depends, first of all, upon contamination with a food-poisoning strain of staphylococcus. The initial number of these organisms or the amount of contamination is important. If the food is heavily contaminated from the start, as, for example, raw milk from infected cows' udders, the organisms will be found in greater numbers in a shorter increment of time than if the initial contamination is slight. The competition which the staphylococci may have with other bacteria present in the food is important. One type may overgrow another. In the case of fresh meat, putrefactive bacteria may multiply to such an extent that the product would be obviously unfit for consumption even if food-poisoning staphylococci were present and produced enough enterotoxin to provoke symptoms of food poisoning. On the other hand, the food in question may not be a favorable medium for good growth of the staphylococcus.

The shortest period of time to produce enterotoxin in foods is difficult to determine, unless extensive experiments on human volunteers are conducted. One of the best experiments on this subject was carried out by Barber (12). This investigator found that cream which contained staphylococci was harmless when refrigerated but became toxic when left at room temperature for 5 hours. In this experiment 5 hours were sufficient for the production of enterotoxin. Whether enterotoxin could be formed in less time in the same medium and under the same temperature conditions was not tested but remains a possibility.

In two naturally occurring outbreaks the significance of time and a suitable temperature is well illustrated in foods containing an enterotoxin-producing strain of staphylococcus and in which groups of people ate the same food items but after a lapse of time. In the first outbreak reported by Tipton and Smiley (29) sliced ham was served at noon mess on one day and 50 cases of food poisoning occurred 4 hours after eating. The leftover ham from this meal was ground up, placed in the refrigerator, and served the following morning with scrambled eggs. One hour after breakfast 300 cases of food poisoning occurred. It is obvious that the shorter period of time from eating the implicated food to the onset of illness indicates a more potent enterotoxin, and this was further correlated with much more severe symptoms. The second outbreak (68) occurred in upstate New York in an industrial plant and was traced to contaminated cream pie. The first person who became ill ate a portion of the pie about 5:30 P.M. and was ill 3½ hours later. Those who ate the pie 9½ hours later (3 A.M.) became very ill within 3 hours after eating. Examination of the pie revealed large numbers of *S. aureus*. In the second outbreak, as in the first, more enterotoxin was produced, which probably followed increase in growth of the staphylococci in the food.

In laboratory experiments with a most favorable medium, as reported by Segalove and the author (49), experiments were carried out to determine the shortest interval of time necessary for enterotoxin production. A good enterotoxin-producing strain of staphylococcus was used. Monkeys were fed cultures prepared as previously described (p. 94). If feeding experiments were negative, monkeys were then injected intravenously with boiled filtrates, which is a more delicate test for enterotoxin. Under these experimental conditions, enterotoxin was demonstrated in a culture grown for 3 days at 18° C. and for 12 hours at 37° C. It was not produced in cultures grown for shorter periods at these temperatures. At 9° and 15° C. it was not produced in 7 and 3 days, respectively. At 4° and 6.7° C. enterotoxin was not formed after 4 weeks. The failure of the staphylococcus to produce enterotoxin at the lower temperatures may be ascribed to poor growth. It may be concluded, therefore, that, among food-poisoning strains of staphylococci, enterotoxin production is a function of growth.

Effect of penicillin on growth and toxin production by enterotoxic staphylococci.—M. Segalove (69) studied the natural resistance of 15 enterotoxic strains which were found to have a wide range of resistance to penicillin (0.05–500 units per milliliter), while five nonenterotoxic strains were found to be relatively sensitive (0.05–0.1 unit per milliliter) to the antibiotic. The addition of penicillin to media did not inhibit enterotoxin production when good growth occurred.

Experimental production of enterotoxin in foods.—Strains of food-poisoning staphylococci have been inoculated into foods and subsequently tested by feeding these to human volunteers. These experiments are outlined in Table 10.

Experiments with fresh beef liver were made by the author in conjunction with Dr. W. A. Starin at Ohio State University and are reported here in detail, since they have not appeared in the literature elsewhere. Two livers were received in a fresh state within a few hours after the animals had been slaughtered. One weighed 4,250 gm., and the other 7,050 gm. The purpose of the experiment was to keep them for the same time and under the same temperature conditions as they would be kept in the modern butcher shop. Before storage they were heavily inoculated inside and outside with a food-poisoning strain of staphylococcus, which had been isolated in 1935 from tongue (70) used for sandwich filling and was known to produce potent enterotoxin. A suspension containing 3.3 billion bacteria per milliliter was injected into each liver. The livers were then placed in a large pan and put into an electric refrigerator. As may be seen in Table 10, they were left in the refrigerator at a temperature of 3.3° C. for 3 days, were wrapped in three thicknesses of ordinary brown wrapping paper, and were allowed to stand in a cool room (16.6° C.) for a period of 6½ hours. This procedure represented the conditions in the butcher shop. They were then placed in an electric refrigerator for 6½ hours and were afterward unwrapped and allowed to stand at a room temperature of 23.3° C. for 50 minutes before cooking, as might happen in the home of the consumer. After the above treatment, the liver was sampled for a bacteriological count and for enterotoxin. With a sterile knife, two wedge-shaped sections were cut through the long por-

TABLE 10

GROWTH AND ENTEROTOXIN PRODUCTION BY STAPHYLOCOCCI INTRODUCED INTO FOOD

FOOD	TREATMENT	INCUBATION (HOURS)	TEMPERATURE (° C.)	BACTERIAL COUNT PER GM.	KIND OF BACTERIA	HUMAN FEEDING	
						Amount (Gm.)	Result
Custard cake filler (71)	None	None	....	610,000	Air contaminants (no staphylococci)	30	Negative
Custard cake filler (71)	None	22	24.4–30	High	....	30	Negative
Custard cake filler (71)	Inoculated with staphylococcus	None	....	414 million	Mostly staphylococci	30	Negative
Custard cake filler (71)	Inoculated with staphylococcus	22	24.4–30	430 million	Mostly staphylococci	5 15	Slightly toxic Toxic
Bread in lettuce sandwich (15)	Inoculated with staphylococcus	5 16 6*	37 6 37	1,100 million	Mostly staphylococci	63	Toxic
Home-baked ham in lettuce sandwich (15)	Inoculated with staphylococcus	5	37	30 million	Staphylococci	Ham from 1 sandwich	Negative
Home-baked ham in lettuce sandwich (15)	Inoculated with staphylococcus Inoculated with staphylococcus	5 16 6*	37 6 37	1,000 million	Staphylococci	48	Toxic
Fresh beef liver	Inoculated with staphylococcus	None	....	2.3 million	Mostly staphylococci	....	Not tested
Fresh beef liver	Inoculated with staphylococcus	72 6.5 6.5 0.83*	3.3 16.6 5.0 23.3	1.5 million	Staphylococci	100	Negative
Canned corn (72)	Inoculated with staphylococcus	168	37	Growth occurred	Staphylococci	30	Toxic
Canned salmon (72)	Inoculated with staphylococcus	96	37	Growth occurred	Staphylococci	90	Negative
Canned oysters (72)	Inoculated with staphylococcus	72	37	Growth occurred	Staphylococci	100	Toxic

* One test was made on this material at the end of the series of incubations.

tion of the large liver, and, in addition, a similar section was cut from the smaller liver. One hundred grams of this raw liver were eaten by a human volunteer who had previously been shown to be susceptible to staphylococcus enterotoxin. He experienced no ill effects. This experiment demonstrates that staphylococcus food poisoning will not occur in liver under the storage conditions in a butcher shop, even if large numbers of enterotoxin-producing staphylococci are present. This work is in agreement with the previously mentioned experiments (49) on time and temperature requirements for the production of enterotoxin by staphylococci.

Certain foods may support the growth of staphylococci without the production of potent enterotoxin, as, for example, canned salmon (72).* Others, such as cured meat products and custard-filled bakery goods, allow food-poisoning staphylococci to grow well and to produce enterotoxin. Aside from such empirical knowledge, however, little is known of the chemical nature of enterotoxin or the chemistry of the metabolism of enterotoxic staphylococci. Staphylococcus enterotoxin is formed irregularly by some strains in veal-infusion broth in flasks incubated aerobically. The difference cannot be explained on the basis of the medium employed, because variations are observed when the same batch of medium and the same aged culture for the inoculum are used. When strains are inoculated into semisolid agar and incubated in an atmosphere containing 10–20 per cent carbon dioxide, a more potent enterotoxin is produced with greater regularity. Thus it seems reasonable that certain foods are more suitable than others for the production of enterotoxin.

On account of the fact that a great many outbreaks of staphylococcus food poisoning have been traced to bakery goods, some workers believed that starch (73) might be of significance, others that protein (62) might be the important factor. Jordan and Burrows (73) found that bacteria of various kinds, i.e., staphylococci, streptococci, *Proteus*, *Bact. coli.*, *Bact. aerogenes*, and *Salmonella aertrycke*, grown under suitable conditions, especially on starch medium, were capable of producing a substance which caused gastrointestinal reactions in monkeys. By successive transfers on starch media, they further succeeded in restoring the enterotoxic property to strains which had lost the ability to produce the substance. They claimed that bacteria which had never possessed this characteristic before could produce the toxic material when grown on starch medium. Others (74) have not been able to confirm the work of Jordan and Burrows. Filtrates were prepared from twelve different kinds of bacteria (74) which had been grown in media with and without starch. The starch-culture filtrate and the corresponding control filtrate without starch were fed in triplicate to monkeys. These animals were observed continuously for a period of 5 hours and at intervals of 8, 12, and 24 hours after feeding, but no symptoms of food poisoning were observed. At the conclusion of these experiments, similarly prepared filtrates from a known food-poisoning staphylococcus strain were fed, and 4 of the 6 animals vomited and showed typical symptoms of food poisoning.

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* In unpublished experiments by M. J. Surgalla and K. E. Hite, extracts of canned salmon experimentally inoculated with enterotoxic staphylococci induced vomiting in 7 out of 51 instances when fed to monkeys.

Properties of enterotoxin: antigenicity.—Staphylococcus enterotoxin is antigenically unlike many bacterial exotoxins, since immunity is irregular and toxin-antitoxin neutralization in multiple proportions has not been demonstrated. Barber makes the following statement relative to immunity:

> Persons who had used the milk continuously had apparently developed some tolerance to the toxin. Two children of the family had used the milk regularly, but never had attacks. The adults had occasional light attacks, or, in one or two cases, some chronic intestinal trouble. In my own case, 4 acute attacks, 3 of them severe, afforded no protection against a subsequent fifth dose. Visitors at the farm and Filipino employees who used the milk less regularly showed most severe attacks. Since the discontinuance of the use of raw milk from this cow, all trouble has ceased [12].

In experiments (33) in which 4 human subjects were fed gradually increasing doses of staphylococcus filtrates from veal-infusion broth cultures grown in a normal atmosphere, in amounts reaching 25–32 ml., a tolerance appeared to develop to the enterotoxin. The tolerance was not high, since, at the conclusion of the experiment, a heterologous filtrate fed to 3 of the 4 volunteers produced illness in 2. Serum from "immunized" men (33) and rabbits did not protect monkeys or rabbits when it was mixed with potent filtrates and injected intravenously, nor did it protect a human volunteer who swallowed it mixed with potent filtrate. The lack of protection after ingestion of a mixture of antiserum and filtrate may, however, not be significant, since the two may be dissociated in the gastrointestinal tract.

Hemolytic, dermo-necrotic, and lethal toxins are the same antigenically for different strains and may be neutralized by a common antitoxin. A nonenterotoxic strain has been used to produce an antitoxin (42) which will neutralize any of the toxins produced by enterotoxic strains except the enterotoxin. Dolman, Wilson, and Cockcroft (44) found that enterotoxin is antigenic, since kittens given several spaced injections of enterotoxic filtrate became resistant, and blood serum from one such kitten neutralized the enterotoxic properties of an equal volume of potent filtrate to such an extent that the mixture proved innocuous when injected into a normal kitten. In our experience (42) such antiserum, when mixed with filtrates containing enterotoxic substances and injected intraperitoneally into kittens, prevented vomiting; but, when the same mixture was injected intracardially into kittens or intravenously into monkeys, food-poisoning symptoms occurred.

If normal kitten blood is added to a mixture of enterotoxic filtrate and antiserum and then injected intraperitoneally into normal kittens, protection is not assured (42). Therefore, the enterotoxic substance is not neutralized either extravascularly or intravascularly in the presence of whole blood by antiserum prepared against such strains. Furthermore, monkeys immunized with a filtrate containing enterotoxin are protected against enterotoxin given by the oral route but not against enterotoxin given intravenously. Monkeys immunized with nonenterotoxic staphylococcus filtrates are not protected against enterotoxin given orally.

Hammon makes the following statement: "Although much has been written regarding the immunological aspects of enterotoxin, there is little in the way of convincing evidence for its antigenicity" (48). To say that enterotoxin is not antigenic is unreasonable, in view of the fact that antiserum prepared against enterotoxin is effective in preventing vomiting when the two are mixed and injected intraperitoneally into kittens. The enterotoxin is, however, unlike other bacterial exotoxins.

Effect of storage of enterotoxin in the refrigerator.—Staphylococcus filtrates containing enterotoxin have been demonstrated to be toxic after storage for as long as 67 days in an electric refrigerator (75). The tests were made by feeding human volunteers 5-ml. portions of filtrates from veal-infusion broth cultures grown in a normal atmosphere. In view of the individual differences in susceptibility in human volunteers, it is uncertain whether a slight loss of potency might not have occurred during the storage period.

Effect of heat.—Enterotoxin which was produced by growing a strain of staphylococcus in beef-infusion broth incubated aerobically was boiled for 30 minutes. When 2 and 10 ml. were fed, respectively, to 2 human volunteers, typical symptoms occurred in both (75). Blood was present in the vomitus and stools of the individual receiving 10 ml. Subsequently, the effect of heat on enterotoxin (76) was studied in monkeys. Filtrates were prepared from 3-day cultures grown on semisolid veal-infusion agar in an environment of 20 per cent carbon dioxide. The medium was strained through gauze, and the fluid which was expressed was centrifugalized to remove most of the organisms and then passed through a Berkefeld "N" filter. This filtrate was subjected to the temperatures given in Table 11 and injected intravenously into monkeys in doses of 0.2, 0.5, and 1.0 ml. per kilogram of body-weight. It is evident from the results in Table 11 that enterotoxin is extremely heat-resistant but gradually loses its potency with prolonged boiling or autoclaving. The rate of destruction is difficult to quantitate, however, since susceptibility to the enterotoxin in both man and animals varies and animals may develop a tolerance to the enterotoxin.

Effect of chlorine.—Chlorine in unpalatable amounts is not effective in destroying enterotoxin. Thus, 0.1 ml. of 0.0915 per cent chlorine solution was added to 5 ml. of staphylococcus filtrate containing enterotoxin and allowed to stand for 3 minutes at room temperature, after which the chlorinated filtrate was added to one-half pint of milk and swallowed by a human volunteer. Typical symptoms occurred in 5¼ hours (75).

Effect of acid and alkali.—There have been some discrepancies in the literature in regard to the effect of acids on enterotoxin. Minett (43) treated boiled filtrate with hydrochloric acid to bring the pH to 5.0 and incubated the filtrate at room temperature and also at 37° C. In one case enterotoxin did not resist 1 week at room temperature, although it resisted overnight exposure at 37° C., whereas in a second case the filtrate was still active after 1 week at room temperature. Hammon (48) lowered the pH of a culture of enterotoxin to pH 4.5 and found active enterotoxin in the supernatant fluid after an incubation for 24 hours at 37° C. Similar treatment with sodium hydroxide to bring the filtrate to a pH of 8.0 or 8.2 appeared to have no deleterious effect (48).

TABLE 11

Treatment	No. Monkeys	No. Positive	Per Cent Positive
Boiled 20 min.	15	4	27
Boiled 30 min.	21	7	33
Boiled 60 min.	14	2	14
Autoclaved 20 min. (120° C., 15 lbs. pressure)	14	1	7

From these studies it would appear that enterotoxin is resistant to acids and alkalies in the amounts indicated.

Effect of formalin.—Filtrates of staphylococci were treated with formalin, so that a final concentration of 0.3 per cent was attained. After incubation for 36 or 48 hours at 37° C., the formalinized filtrates produced vomiting in kittens. In another experiment, however, enterotoxin was inactivated by 0.3 per cent formalin after 10 days at 37° C. (43).

Effect of chloroform.—Jordan and Burrows stated that the enterotoxic substance "is completely removed from acid aqueous solution by extraction with ethyl ether or chloroform" (77); but they were not certain whether the enterotoxic substance was really dissolved or merely adsorbed on the chloroform globules, since the preparation that they fed to monkeys was an emulsion of chloroform and filtrate (77). To test this point, Davison and the author (76) extracted a filtrate containing enterotoxin with three changes of chloroform and then broke up the fine emulsion of chloroform and filtrate by centrifugalization for an hour. After the chloroform had been removed from the fractions by evaporation in a strong current of air at room temperature and by storage in an incubator overnight, the residue was taken up in olive oil or suspended in saline. No vomiting was obtained in monkeys fed the extract taken up in olive oil or in kittens injected intracardially with the extract in saline. The supernatant fluids when fed to monkeys or when boiled and injected intracardially into kittens caused vomiting.

Effect of ether.—Hammon (48) was unable to find any active enterotoxin in ether extracts but found it in the residual culture filtrate.

Solubility in alcohol.—Davison and Dack (76) treated enterotoxic filtrates over a wide range of pH with two different concentrations of ethyl alcohol. Fractions were precipitated with 50 per cent and also with 76 per cent alcohol at a pH of 6.8, 7.2, and 8.0. In each case the mixture was refrigerated overnight, and the resulting alcoholic precipitates and filtrates were assayed by intravenous injections into kittens. Although conditions of pH, time, temperature, etc., were controlled, the results obtained at one time were not always duplicated at another. Hammon (48) has been able to precipitate enterotoxin with ethyl alcohol repeatedly, both from crude casein hydrolysate toxin and from infusion-broth toxins. The enterotoxin was first dialyzed with 0.8 per cent sodium chloride as an electrolyte. One volume of the dialysate containing the enterotoxin was then treated with 2 volumes of 95 per cent alcohol, which precipitates the enterotoxin. The alcohol toxin was stored at 2° C. for 24–48 hours, and the sediment was collected after centrifugalization and redissolving in normal saline solution. It is evident from these two reports that staphylococcus enterotoxin may be precipitated under certain conditions with alcohol and was not appreciably denatured in the process.

Precipitation with ammonium sulphate.—Davison and Dack (76) found that enterotoxin was consistently "salted out" from saturated solutions of ammonium sulphate but not from half-saturated solutions. Hammon reported the other toxins of staphylococci are precipitated with 75 per cent ammonium sulphate, whereas the enterotoxin remained in solution.

Dialysis of enterotoxin.—Jordan and Burrows (77) found that the active principle of enterotoxin is not readily dialyzable. Minett (43) reported that enterotoxin diffuses only slightly through collodion. Davison (72) states that enterotoxin will pass through a collodion membrane of 3.5 mμ pore size. Since a particle passing a small membrane is one-third to one-half the size of the pore, according to Elford (78), the upper limit of molecular size of the enterotoxin is about 1.7 mμ.

Effect of proteolytic ferments on enterotoxin.—Minett (43) found that enterotoxin was resistant to rennet but was destroyed by trypsin. Davison (72) reported that it was destroyed by pepsin. On the other hand, Hammon (48) reported that enterotoxin was resistant to both trypsin and pepsin. The reason for the lack of agreement in this field is not apparent. Although pepsin usually attacks molecules of considerable size, workers (79) have recently found a few simple compounds which can be split, and these exceptions eliminate the possibility of computing protein complexity by peptic digestion. Hammon makes the following statement regarding the chemical nature of enterotoxin:

> Small amounts of material originally prepared in a simple, protein-free medium, and which had undergone considerable purification by fractionation with ammonium sulfate and alcohol, were shown to be active, yet, in the concentration used for inoculation, contained no nitrogen detectable by the micro-Kjeldahl method and did contain a reducing carbohydrate substance. It seems probable from this evidence that the active substance is in the form of a large, complex, carbohydrate molecule, and is not a protein [48].

If the chemical composition of enterotoxin were known, not only might some chemical test be devised for detecting it, but our knowledge of staphylococcus food poisoning would probably progress rapidly.

Mode of action of enterotoxin.—The mode of action of staphylococcus enterotoxin is not understood. M. Bayliss (80) has studied this problem in the cat. He found no direct action of enterotoxin on small strips of intestine from freshly killed cats or rabbits when suspended in Ringer's solution. Emesis followed intravenous, intracardial, or intraperitoneal injections but failed to appear subsequent to oral, subcutaneous, or intramuscular administration. Emesis occurred when enterotoxin was administered following surgical procedures, such as removal of the celiac ganglion, removal of the stomach, severing of the spinal cord at the level of the second or lower thoracic vertebra, and severing of one of the vagus nerves. Following such surgical procedures as severing both vagus nerves, abdominal evisceration, and severing the spinal cord at the level of the seventh cervical vertebra, mild retching movements and, rarely, emesis occurred when enterotoxin was injected. Emesis never occurred after destruction of the vomiting center in the brain, injection of enterotoxin into the fourth ventricle over the vomiting center, or severing the brain between the anterior border of the pons and the posterior border of the hypothalamus. Morphine inhibited, ergotoxine inhibited or delayed, whereas atropine had little or no effect on emesis due to the injection of enterotoxin. Bayliss interprets his experiments as follows: ". . . The action of staphylococcus enterotoxin on peripheral sensory structures is of greater importance in the initiation of emesis than direct action of enterotoxin on the vomiting center" (80). Although Bayliss' experiments were controlled, the amount of surgery and trauma involved in them makes further work seem desirable to establish the mode of action of the enterotoxin.

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CHAPTER VI

Salmonella

PERHAPS no field is more confused than the one concerned with the role of the *Salmonella* organisms in food poisoning. *Salmonella* is a generic name applied to a group of bacteria which were formerly called "paratyphoid bacteria." They were named for D. E. Salmon, who, with Theobald Smith (1), first described a member of the group. There are many reasons why members of *Salmonella* are so often suspected in outbreaks of food poisoning. The most important of these is: *Salmonella* have been known to be associated with food poisoning for so many years when other types were unrecognized that, until recently, bacteriologists automatically suspected members of the *Salmonella* group if *Clostridium botulinum* could not be implicated. Theories and conclusions have been advanced which (as will be discussed later) have falsely held that heat-stable products of *Salmonella* when ingested by man would give rise to symptoms of food poisoning. Therefore, it was not always considered necessary to recover the organism in culture. *Salmonella* were often thought to be isolated but turned out to be, upon thorough study, a slow lactose fermenter, belonging to the coliform group. Also, a positive diagnosis of *Salmonella* food poisoning might be falsely based upon a low titer of *Salmonella* agglutinins in the serum of patients. Thus many outbreaks have been reported as caused by *Salmonella* in which these microörganisms have had no part.

CHARACTERISTICS OF *Salmonella* OUTBREAKS

The *Salmonella* group of bacteria is composed of many species, and new ones are still being described. In general, they cause two types of infection. One group apparently parasitizes only man and produces an infection and symptoms similar to, but milder than, typhoid fever. To this group belong the species *Salmonella paratyphi* and *S. schottmülleri*. Man may become a carrier of these organisms.

The second group produces in animals a disease similar to typhoid fever in man and, if ingested in large numbers by man, produces one or more of the following symptoms: nausea, vomiting, abdominal cramping, and diarrhea. Frequently, fever and leukocytosis are also present. The first outbreak of this type of food infection to be described occurred in the village of Frankenhausen in Saxony, Germany, in 1888. In this outbreak, described by A. Gärtner (2), 57 persons became suddenly ill with symptoms of acute gastroenteritis. One young man consumed 800 gm. of raw meat and died in about 35 hours. From the organs of this fatal case, Gärtner isolated a microörganism which he called *Bacillus enteritidis*. He isolated the same organism from the meat of the diseased cow which was responsible for the outbreak.

Another interesting episode was described by E. van Ermengem (3), in which several men ate sausages that were alleged to have been responsible for illness. A sanitary inspector of meat in a village of Belgium, who was reported to be a very famous veterinarian (M. L.), examined the incriminated sausages. Reassured by the excellent appearance of the sausages and their good odor and color, the expert did not hesitate to declare them fit for consumption and volunteered to eat some of the sausages to prove his absolute conviction of their perfect harmlessness. M. D. K., the director of the slaughterhouse, and three workers also ate some of the sausages. M. L. ate three slices of one of the sausages at 11:00 A.M. on Saturday, October 26, 1895. After 10–12 hours he developed a severe diarrhea and accompanying weakness, which continued and increased in severity until on Monday, when he called his physician, he had continuous diarrhea, violent colic, epigastric distress, a pulse of 110, and a temperature of 102° F. On Tuesday the diarrhea persisted, with a foul-smelling yellowish discharge. The patient was restless and had precordial pain. The abdomen was not bloated;

the urine was dark and contained a large quantity of albumin. There was manifest mydriasis, and the pupils reacted to light and in accommodation. The pulse on Tuesday was 100 and the temperature 101.5° F. On Wednesday morning M. L. was delirious and prostrated, with a temperature of 95.5° F. The stools were involuntary. The spleen and liver were enlarged. On Thursday the temperature was 97.9° F., and there was marked precordial pain. Thereafter the patient's condition became progressively worse, and he died at 6:00 A.M. Friday morning—about 6 days after eating the sausage. M. D. K. ate three or four slices of the sausage at about the same time as did M. L. He said it had no abnormal taste but was strongly salted and spiced and seemed to have an aftertaste that was metallic, although M. L. had not detected this. On Sunday morning M. D. K. had diarrhea, with five to six stools, i.e., 20–24 hours had elapsed between the eating of the sausage and the onset of symptoms. At this time there was no colic, but the patient had a general feeling of illness. Diarrhea continued in the afternoon, with stools about every half-hour. The appetite was poor. The next morning (Monday) stools were less frequent, but the abdomen was tender, headache and lumbar pain were present, the pupils were dilated, and the vision was blurred. On the following day (Tuesday) his urine was abundant, turbid, and brownish, verging on red. The stools were yellowish and fetid. Vision had returned to normal. Thereafter symptoms gradually abated, and recovery followed in about a week. The accounts of the three workers were sketchy and incomplete. One who ate two slices of sausage became ill in 28 hours, with fever, headache, general lassitude, frequent diarrhea, thirst, etc. The second tasted the sausage but experienced nothing more than some abdominal discomfort, for which he took a laxative. The third worker ate one slice of three different sausages given him by M. L. but had only a slight headache.

A necropsy was performed on M. L., and a diagnosis was made of acute gastroenteritis, probably infectious, with congestion and degeneration of the liver, acute parenchymatous nephritis, and pulmonary hyperemia. Typical *S. enteritidis* was isolated from the liver, spleen, lung, kidney, muscle (not so many colonies), portal blood (few), and the contents of the ileum. A mixture of liver and kidney from M. L. was injected subcutaneously, intraperitoneally, and by stomach tube into guinea pigs, rabbits, mice, rats, and dogs. All died in a short time except two dogs which had been fed the material. The animals were examined immediately after death, and *S. enteritidis* was recovered from their livers, kidneys, and other tissues. It is obvious, therefore, that M. L. had a septicemic infection, with the strain occurring naturally in animals. Not only was *S. enteritidis* isolated from the sausages, but microscopic and chemical tests were negative for other agents or chemical poisons.

The outbreak described by Van Ermengem illustrates many important points. In the first place, not all individuals react similarly, and the severity of symptoms is not proportional to the quantity of meat eaten. In the case of M. L., both the gastrointestinal and the septic types of disease were produced, whereas in the other four only the gastrointestinal symptoms were manifested, being extremely mild in three of the four. In the second place, 12–24 or more hours passed from the time of eating the contaminated food to the first appearance of symptoms. This interval of time provides an opportunity for *S. enteritidis* to multiply in the body and to invade the tissues.

INCUBATION PERIOD

One of the most characteristic features of *Salmonella* gastrointestinal infections is the time required for symptoms to develop after the ingestion of the organisms. This particular point is not stated clearly in most textbooks, because, in reports published before staphylococcus food poisoning was generally recognized, food-poisoning outbreaks in which the incubation period was 2–3 hours were often ascribed to *Salmonella*. Present knowledge makes it possible to state that such outbreaks were, in all probability, due to staphylococci. Also characteristic is the variability in the time at which symptoms appear. The symptoms result from infection rather than from intoxication by pre-formed toxins; hence the incubation period varies with the time required for the multiplication and invasion by the organism in individuals with differing degrees of resistance.

In Table 12 a list of some carefully studied *Salmonella* outbreaks illustrates the length of the incubation period. In all these the organisms were isolated from the patients and in some instances from the food also. From this table, involving 482 cases, it may be seen that a period of at least 5 hours elapses before symptoms develop in the most susceptible individuals, 12–24 hours elapse in the majority of cases, and 24–30 hours or longer may elapse in a few cases. Finally, the fatalities are not high. Out of the 482 cases, only 4 died.

TABLE 12

CHARACTERISTICS OF *Salmonella* GASTROINTESTINAL INFECTIONS

Outbreak	No. Cases	Causative Organism	Food	Time from Swallowing Organism to Onset of Symptoms (Hours)	Deaths
1 (4).......	18	*S. enteritidis*	Bread pudding	8–20	None
2 (5).......	135	*S. enteritidis*(?)	Milk contaminated with rat virus	12–48 (Peak 24–30)	None
3 (6).......	150	*S. cholerae-suis*	Milk	8–36	None
4 (7).......	65	*S. typhi-murium*	Cheese	21–42	1
5 (8).......	31	*S. enteritidis*	Mutton	15–72	2
6 (9).......	52	*S. typhi-murium*	Rice pudding made with infected duck eggs	7 and longer	1
7 (10)......	20	*S. oranienburg*	Scrambled egg from dried eggs	12–40	None
8 (11)......	9	*S. berta*	Pork sausage	5–48	None
9 (12)......	1	*S. senegal*	Aspirated culture into mouth while pipetting	20	None
10 (13)......	1	*S. saint-paul*	Accidentally sucked suspension into mouth through unplugged pipette	48	None

SYMPTOMS

The symptoms of *Salmonella* gastrointestinal infection are characterized by nausea, vomiting, abdominal pain, and diarrhea. The onset is usually sudden. The attack may be ushered in with headache and a chill. The abdominal pain is often the first symptom, and it may be griping and severe, together with persistent, foul-smelling diarrhea. Later in the attack the stools may be watery and sometimes of a greenish color. Prostration, muscular weakness, faintness, and thirst are marked. There is almost always a rise in temperature, with various nervous manifestations of restlessness, muscular twitchings, and drowsiness. Oliguria may be present, and herpes frequently follows.

The severity of the disease differs in different outbreaks and among various individuals in the same outbreak. Dosage or number of organisms ingested may be an important factor, since, in some experimental work as well as in outbreaks, individuals who have had a small dosage may escape without symptoms. All *Salmonella* organisms causing gastrointestinal upsets in man, however, seem to cause identical symptoms and to run the same course, regardless of the species involved. In a small percentage of the cases the organisms may invade the tissues and cause death.

LABORATORY DIAGNOSIS OF *Salmonella* AS THE CAUSE OF GASTROINTESTINAL UPSETS

The best diagnosis can be attained when *Salmonella* are found in both stools and suspected food. Unfortunately, this is not often possible. According to E. Verder and C. Sutton (14), the smaller the number of organisms swallowed, the longer is the incubation period, but during the time of acute symptoms practically pure cultures of *Salmonella* may be recovered—a fact which indicates that *S. enteritidis* may supplant the normal fecal flora. Thereafter, the organisms rapidly disappear from the stools. Often outbreaks are not studied until a day or more after acute symptoms have subsided, and then the causative bacterium is not always present. Suspected foods may be ground with sterile sand in a sterile mortar and streaked on appropriate media, but the incriminated foods are frequently not available for test.

There are several selective media available for culturing *Salmonella*. In the author's experience, the *Salmonella-Shigella* medium, manufactured in dehydrated form by the Digestive Ferments Company, has been found to be excellent. Since *Bacterium coli* is to a great extent inhibited on this medium, *Salmonella* organisms may be found even when they are present in small numbers in feces. It is, however, a good plan to use some other less inhibitive media in the event that an unusual *Salmonella* strain may be inhibited by the *Salmonella-Shigella* medium. The probability of isolating *Salmonella* organisms, when present in small numbers, may be aided considerably by preliminary enrichment methods of appropriate samples in such media as tetrathionate broth, or selenite F medium (15). Subinoculations may then be made into differential media.

The technical methods of identifying suspicious nonlactose fermenting colonies of bacteria occurring on plated media are sufficiently described in textbooks of bacteriology to warrant omission in this treatise, but differential characteristics are discussed on pages 136–39. Since the final serological tests to determine antigenic structure require a number of strains and antisera which most laboratories do not possess, it is advisable to send cultures to a laboratory that is equipped for this work (cf. p. 136).

W. G. Savage and P. B. White (16), in studying cases of food poisoning in which *Salmonella* were suspected but no living organisms could be found in the food, tested for the presence of specific toxic substance by the following three methods: (1) the production of gastrointestinal irritation in rabbits fed suspected food; (2) the demonstration of specific agglutinins in patients' serum; and (3) the injection of animals with extracts of the suspected food and the demonstration of specific agglutinins in their serum. The discussion which follows indicates, however, that none of these methods is reliable.

The first method consisted of feeding suspected food to young rabbits, killing them in 9–12 hours, and examining their stomach and duodenum. Post mortem examination showed various degrees of hyperemia of the mucosa, with little correlation between severity of hyperemia and dosage, strain, etc. The method of killing the rabbits was not reported. No controls, fed other materials and killed in the same way after the same interval, were reported. These experiments have been repeated with negative results by G. M. Dack, P. H. Harmon, and J. E. Jarra (17) on 17 rabbits killed by suffocation.

Savage and White state that their second criterion is not very reliable. The agglutination titers were all very low (only two above 1:100) and in many cases were negative. Where there was definite agglutination, the titer varied considerably from one day to the next. They explain the lack of agglutinins in some patients by the rapid expulsion of antigen, owing to the diarrhea and vomiting, and in others by the ingestion of the toxic food after it had been well cooked. (The same authors could demonstrate no agglutinins in laboratory animals which had been fed boiled cultures.) Similarly, the experience of others has indicated that the agglutinin titer in the serum of individuals experiencing an attack of *Salmonella* infection of the food-poisoning type is frequently not very high. M. J. Rosenau and H. Weiss (4) tested the serum of 3 patients, about 10 days after an attack, with the specific organism isolated from food. They found agglutination occurring in a dilution of 1:100 of patient's serum but not in 1:500. A. R. Culley (8) tested serum from 29 patients 10 days after an attack and in 19 found titers ranging from 1:25 to 1:500. The remaining 10 serums from patients with mild attacks did not agglutinate the specific strain in a 1:25 dilution. Verder and Sutton (14) tested the serum of a volunteer who was made ill with *S. enteritidis* at the time of illness and 10 days after recovery. At the time of illness the specific organism was agglutinated only partially in 1:20 and 1:40 dilutions, whereas 10 days later there was complete agglutination in 1:20 and 1:40 dilutions and partial agglutination in dilutions of 1:80 and 1:160. Moreover, monkeys which had been fed living *Salmonella* cultures (18) and had developed gastrointestinal symptoms characteristic of those occurring in outbreaks in man did not produce an appreciable agglutinin titer to the specific organisms fed. It is obvious, therefore, that the agglutinin titer for the organisms causing *Salmonella* infections of the food-poisoning type is not increased sufficiently to be of definite diagnostic value in man or monkeys.

The third of Savage and White's criteria is not valid because the results can be interpreted differently. Owing to the prevalence of *Salmonella* infections in domestic animals and rodents, the tested animals may have a low titer of antibody because of previous slight infections. This titer may increase after the injection of a foreign antigen, such as a suspected food, since the intravenous injection of any antigen increases the titer of all antibodies present in the serum (anamnestic phenomenon). No titer reported by Savage and White (16) was higher than 1:200, the majority was much lower, and no control with bland protein was recorded.

As stated previously, it is necessary to demonstrate living *Salmonella* in specimens submitted for bacteriological examination, in order to substantiate a diagnosis of *Salmonella* infection of the food-poisoning type.

CONTROL OF *Salmonella* INFECTIONS OF THE FOOD-POISONING TYPE

Although *Salmonella* infections account for only a small number of food-poisoning outbreaks, they frequently arouse public concern. In attempting to control these infections, it is important to understand the sources and modes of infection.

Members of the *Salmonella* group cause enteric and systemic infections of animals. As previously mentioned, when cattle with these infections are slaughtered and the meat is eaten in an undercooked condition by man, illness results. The problem of preventing outbreaks spread in this manner is one of public health interest.

E. Hormaeche and R. Salsamendi (19) studied the *Salmonella* infections in children in Montevideo, Uruguay. In searching for the source of these infections they examined the mesenteric lymph glands of apparently normal hogs slaughtered for the market. They examined 46 lots of hogs, consisting of 20 animals each, and found that 47.9 per cent yielded *Salmonella* organisms. H. L. Rubin, M. Scherago, and R. H. Weaver (20) cultured the mesenteric lymph glands from 40 lots of hogs, consisting of 25 animals each, and found 47.5 per cent of the lots contained *Salmonella*. Of 50 hogs studied individually, in which the mesenteric lymph nodes were cultured, 5 yielded *Salmonella*. The frequency of the types found by the latter workers is given in the accompanying tabulation.

S. typhi-murium	7
S. cholerae-suis (variety from Kunzendorf)	4
S. oregon	4
S. anatum	3
S. give	3
S. bareilly	2
S. derby	2
S. new brunswick	2
S. bredeney	1
S. enteritidis	1
S. lexington	1
S. newington	1
S. worthington	1

In these experiments (19, 20) enrichment methods of culture were used which are selective and would reflect small numbers of *Salmonella* present. It is doubtful whether the *Salmonella* recovered represented invasion of the lymph nodes, since no controls were made to eliminate surface fecal contamination which occurs normally in slaughtering operations. Only under aseptic measures, such as those used in abdominal surgery, and before the bowel was cut could suitable bacteriological specimens of mesenteric lymph nodes be secured. It may well be, therefore, that the *Salmonella* which were obtained reflect fecal contamination and not these organisms as invaders of the mesenteric lymph nodes. From the public health standpoint there may be a significant difference in the dosage or numbers of *Salmonella* contaminating the meat. In the case of tissue invasion the dosage may be greater than the natural fecal contamination from the carcasses of carrier animals.

W. B. Cherry, M. Scherago, and R. H. Weaver (21) examined various types of meat products from retail markets by means of a cultural enrichment method for isolating *Salmonella* species. Of 250 samples examined, 13, or 5.2 per cent, were found to contain *Salmonella*, and the incidence in pork products was greater than in beef. Again this study may reflect the small amount of fecal contamination incident to slaughter. As our knowledge of *Salmonella* species in nature grows, it does not now appear that the utopia of *Salmonella*-free domestic animals will be reached. The low incidence of *Salmonella* food-poisoning outbreaks in man would indicate that our methods of handling and cooking meats are satisfactory.

Meat sold in interstate commerce has to be inspected by federal agents. Obviously, animals suffering from *Salmonella* infections are thus eliminated. The United States federal meat-inspection law was approved March 18, 1907, and amended in 1913, 1919, 1922, and 1930. It provides for the inspection of animals the meat or products of which are to enter interstate or foreign shipment. Such animals include cattle, sheep, goats, swine, and, in a restricted way, horses. The law is administered by the Bureau of Animal Industry, United States Department of Agriculture.

The purpose of a meat-inspection service is to prevent the sale of diseased or unsound meat and to see that clean and sanitary conditions are provided for the preparation and processing of foods composed wholly or in part of meat. The use of harmful dyes, preservatives, chemicals, or other deleterious substances is forbidden. False and misleading labels and statements are not allowed. The inspection service carried out by the Bureau of Animal Industry is divided as follows: (1) sanitation; (2) ante mortem, or live-animal, inspection; (3) post mortem, or slaughter, inspection; (4) products inspection, or supervision of processing and manufacturing meat food products; and (5) laboratory inspection.

In regard to sanitation, meat-storage places must be ratproof. On the killing floors the equipment used in slaughter must be properly sterilized, so that knives or other apparatus infected with contaminated material will be free from contamination before they are used on sound carcasses. A pure water supply and good drainage must be provided. The ante mortem inspection eliminates most of the sick animals. Under this inspection, animals sick with *Salmonella* infections are detected and thus not sold as food. Such an outbreak, as described by Gärtner (2), in which a sick cow was slaughtered and the meat salvaged and sold, could not have occurred if the animal had been properly inspected. The evidences of *Salmonella* infection may be recognized by the experienced meat inspector, and diseased animals that may get past the ante mortem inspection are discovered and condemned at post mortem inspection. The final inspection also guards against contamination of the products in the course of manufacture (curing, etc.) after slaughter. The scarcity of *Salmonella* outbreaks from meat products in this country is probably a true measure of the effectiveness of our meat inspection. Our excellent federal meat inspection has been an example to municipalities which have to deal with small abattoirs furnishing meat that is consumed locally.

Meat poisoning, instead of having the usual indefinite meaning, to many German workers refers specifically to *Salmonella* infection of the food-poisoning type, although food poisoning by bacteria other than *Salmonella* has been caused by contaminated meat. Many outbreaks of *Salmonella* infection, however, have been due to foods other than meat (4, 5, 6, 7, 8, 9). Milk and cheese may become contaminated from a diseased cow or subsequently upon handling. Pasteurization of milk is effective in destroying *Salmonella*.

Rats and mice may be a reservoir of *Salmonella* in nature, and natural infections in rodents have often been recognized. This subject is reviewed by M. T. Bartram, H. Welch, and M. Ostrolenk (22). These workers found that fatal infections usually follow the feeding of rats with massive doses of *S. enteritidis*. On the other hand, more uniform and lasting infections are obtained by single, small doses. Thus positive fecal specimens were obtained from 15 out of 24 rats fed 100 organisms, beginning in some instances on the first day and persisting sometimes for as long as 34 days. That rats may be carriers was suggested by the intermittent excretion of the infecting organism over a period of at least 7 weeks. Continued high agglutinin titers were obtained in these animals for at least 4 months after infection. Transmission of the infection from an infected rat to cage mates was demonstrated, with recovery of the infective organism from the composite feces and from individual rats as long as the fortieth day after contact with the infected animal. Examination of feces from 800 white laboratory rats and agglutination tests on the blood of 26 revealed the absence of *Salmonella* infection in all instances. These same workers (23) in a later report found that excreta of rats infected with *S. enteritidis* when held at room temperature may contain living organisms for at least 148 days. They also ascertained that infection of rats and mice with very few organisms is possible when a virulent strain of *S. enteritidis* is fed them by stomach tube. They were able to transfer the infection from an infected animal to cage mates through 7 colonies of rats and through 3 colonies of mice.

A study of the excreta of rats and mice collected in areas throughout the United States indicates that only a small percentage (1.2 per cent) excrete food-poisoning organisms of the *Salmonella* type. Perhaps this small percentage may be attributed in part to the limitations of the laboratory methods used by the authors. They streaked only plates of bismuth sulphite agar from tetrathionate enrichment culture of feces. The incidence of infection in wild rats and mice is reported by these workers as follows:

> The results of examining some 420 specimens of rat and mice pellets, from which only five strains of *Salmonella* of the food poisoning type were isolated, collected throughout the country, however, might explain the dearth of food poisoning outbreaks proved to be the result of infected rodents. It is true that much higher incidences of food poisoning organisms have been reported in rats and mice, but invariably the investigators reporting such figures gathered their animals in a small area, or from a definite source, such as a packinghouse or bakery and in several instances the studies were carried out because of a recent outbreak. Under such conditions with the apparent ease of transmission of infection in rat colonies higher incidences of infection in a given area might be expected [23].

The role of rats in the spread of *Salmonella* infections of the food-poisoning type is summarized by these workers as follows:

> Although a much larger series of specimens of rodent excreta need to be examined from many more areas in the United States before a definite conclusion can be reached, our studies to date indicate that relatively few rodents are infected with food poisoning organisms of the *Salmonella* group. It would appear that this would explain, in part at least, the relatively few outbreaks of food poisoning in which rodents were proved to be vectors of the causative organisms. Nevertheless, since some few rats or mice may be infected with food poisoning organisms, they all must be considered potentially dangerous to health, and every effort should be made to eliminate them from establishments where human food is prepared or stored [23].

Salmonella organisms have been used in an attempt to exterminate rats. The folly of such a procedure is well illustrated not only by the experimental work just reported (22, 23) but by outbreaks which have been directly traceable to this procedure. Spray (5) in 1926 reported an outbreak of 135 cases probably due to milk contaminated with rat "virus." The commercial rat virus was distributed about the kitchen, serving-room, and storerooms 16 days before the outbreak. It lay on the floor, which was mopped daily with a string mop, requiring hand wringing, by one of the kitchen assistants whose other duties included washing dishes and preparing salads. In addition, a bag of the rat-virus bait obtained from the serving-room was found to contain viable bacteria identical with strains isolated from patients.

Salmonella are propagated in the intestinal tracts of all domestic animals, and the dog is no exception. Wolff and his co-workers (24) collected rectal swabs from 100 dogs and recovered *Salmonella* types from 18. They identified the following types: *S. manhattan*, *S. newport*, *S. minnesota*, *S. oranienburg*, *S. typhi-murium*, *S. bredeney*, *S. worthington*, *S. give*, *S. cubana*, *S. cerro*, *S. kentucky*, *S. illinois*, *and S. meleagridis*. In addition, two types were isolated which had not been named. It is obvious that *Salmonella* organisms are widespread in the intestinal tract of animals and that, whenever uncooked foods are contaminated with the excreta of animals or man which are carriers or cases of *Salmonella* infection, there is a potential danger of infecting the consumers of the food.

The housefly (*Musca domestica*) has been shown to be responsible for spreading enteric diseases. During the Boer (25) and Spanish-American wars (26), thousands of cases of typhoid fever were traced to the contamination of food by flies. In an experimental study of the housefly as a vector of food-poisoning organisms in food establishments (27), it has been found that the number of bacteria deposited is determined by the nature of the material on which the flies feed. When *S. enteritidis* was experimentally inoculated on food upon which flies were allowed to feed, the flies were found to infect other flies, as well as food, water, and other surfaces with which they came in contact. *Salmonella enteritidis* could be found in maggots, pupae, and adult flies coming in sequence from contaminated fly eggs. The organism was found to survive in the fly for the duration of its life, approximately 4 weeks. In view of these experiments, it is obvious that flies may play an important role in the spread of food-borne infections and that foods should be protected from them.

Since *Salmonella* organisms may gain entrance to food from rodents, houseflys, and human carriers, it is important to know something of the growth of these organisms in different foods. *Salmonella enteritidis*, experimentally inoculated into test-tube preparations of canned roast beef, corned beef, and potted meat (28) grew luxuriantly and survived for at least 60 days at 22° and 37° C. when loss of moisture was prevented. No abnormal odor occurred in the food. A strain of *S. enteritidis* was experimentally inoculated into cans of asparagus, spinach, string beans, tomato juice, peaches, shrimp, salmon, corn, and peas (29). Duplicate cans of each food were incubated at 22° and 37° C. Growth occurred in asparagus, peas, corn, spinach, string beans, tomato juice, shrimp, and salmon incubated at 22° C. and in peas, corn, spinach, string beans, tomato juice, salmon, and shrimp at 37° C. *Salmonella enteritidis* appeared to grow better and persist longer at 22° than at 37° C. In asparagus, string beans, tomato juice, and peaches viable organisms were found at 22° C. after 30 and 60 days, whereas after 30 days at 37° C. no viable organisms could be detected except in the cans of string beans. *Salmonella*, obviously, are not found in unopened canned foods, since the processing times and temperatures of canning preclude the survival of this organism. Therefore, *Salmonella* food poisoning from canned foods could occur only after the cans had been opened and contaminated with the specific organism. Under natural conditions, pure cultures of *Salmonella* would not contaminate foods, and the multiplication of these organisms in foods would be competitive with other naturally occurring contaminants. Since, in this country, *Salmonella* food poisoning is uncommon, it is obvious that *Salmonella* do not survive for any length of time outside their natural hosts.

THE *Salmonella* INVOLVED IN GASTROINTESTINAL INFECTIONS

The types of *Salmonella* which produce in man disease resembling typhoid fever are not considered here, since their epidemiology is identical with that of typhoid fever.

Forty-three species, comprising 1,490 strains of *Salmonella*, have been isolated from cases or outbreaks of food poisoning in England and Wales from 1923 to 1944 (10). Of this number of strains, 56 were not identified as to species. The 10 most common species arranged in order of prevalence were as follows:

	No. Outbreaks		No. Outbreaks
S. typhi-murium	665	*S. montevideo*	37
S. enteritidis	171	*S. cholerae-suis*	22
S. newport	159	*S. bovis-morbificans*	22
S. thompson	136	*S. dublin*	19
S. oranienburg	76	*S. meleagridis*	19

P. R. Edwards and D. W. Bruner (30) studied 224 cultures (excluding *S. paratyphi* A and *S. paratyphi* B) isolated from the stools of patients with gastroenteritis. The 10 most numerous species in order of importance were as follows:

	No. Strains*		No. Strains*
S. typhi-murium	43	*S. sandiego*	11
S. newport	31	*S. bareilly*	9
S. paratyphi B var. *java*	30	*S. montevideo*	9
S. panama	14	*S. enteritidis*	8
S. oranienburg	13	*S. anatum*	7

* Seven strains of *S. give* were also studied.

Exclusive of *S. paratyphi* A and *S. paratyphi* B, 39 species were studied. One hundred and eighteen cultures from the stools of carriers were studied, and these were scattered among 24 types or species. Twenty-five cultures comprising 7 species were isolated from the stools of patients with enteric fever.

In four other laboratories (31, 32, 33, 34) the prevalence of *Salmonella* species is somewhat at variance with those reported for England and Wales. In all four collections studied, *S. panama* varied from the fifth to the tenth most prevalent species, and in three of the four collections *S. anatum* varied from the third to the tenth most prevalent species. In general, *S. typhi-murium*, *S. montevideo*, *S. oranienburg*, and *S. newport* are the types most frequently encountered.

During World War II, dried eggs were shipped in large quantities to Europe. Of 7,584 samples (10) from the United States, Canada, and Argentina, 754, or 9.9 per cent, were found to contain *Salmonella*. Thirty-three species were identified, and the 10 most common in order of importance were as follows:

	No. Isolated		No. Isolated
S. oranienburg	245	*S. anatum*	30
S. montevideo	139	*S. typhi-murium*	30
S. meleagridis	117	*S. newport*	24
S. tennessee	90	*S. senftenberg*	8
S. bareilly	82	*S. london*	6

Twenty-two of the 33 species, including the 6 most common, had never, so far as could be ascertained from published accounts, been met with before 1941 in cases of human infection in

Great Britain. In 1943 there was a rise in the annual number of food-poisoning outbreaks recognized as being due to *Salmonella* out of all proportion to what might have been anticipated, and in 1944 a further rise occurred. In a study of 428 outbreaks of food poisoning in England between 1923 and 1939, 14 different species were met with and only 9 for a single year. During the latter part of 1942 and during 1943 and 1944, outbreaks of food poisoning occurred which were due partly to the old *Salmonella* species and partly to 24 new species. Of the new species isolated, the 6 commonest in order were the same 6 as headed the list of the dried-egg strains. From the above facts the British concluded that dried egg was probably responsible for a considerable proportion of the greatly increased number of outbreaks of food poisoning that occurred during the years 1943 and 1944.

N. E. Gibbons and R. L. Moore (35) studied Canadian egg powder and isolated *Salmonella* organisms from 28 of 380 samples. *Salmonella* counts were made on 21 samples, and 13 contained less than 1 organism per gram; 7 between 1 and 10; and 1, 54 per gram.

M. Solowey, V. H. McFarlane, E. H. Spaulding, and C. Chemerda (36) isolated *Salmonella* from 35 per cent of 5,198 samples of spray-dried whole egg powder obtained from 100 dehydration plants between September, 1943, and January, 1945. The incidence varied from 0 to 71 per cent for individual plants. Fifty-two *Salmonella* types were identified, 5 of which—*S. pullorum*, *S. oranienburg*, *S. montevideo*, *S. tennessee*, and *S. anatum*—made up 73 per cent of the isolations.

The source of *Salmonella* in egg powder apparently was from infected flocks. W. R. Hinshaw, E. McNeil, and T. J. Taylor (37) studied 561 *Salmonella* strains from 353 avian outbreaks caused by 23 types, 21 of which were types which have also been found in man. *Salmonella typhi-murium* was recovered in 60 per cent of 291 outbreaks in turkeys and 35 per cent of 43 outbreaks in chickens. *Salmonella bareilly* caused 42 per cent of the outbreaks in chickens but only 5 per cent of those in turkeys.

James Watt (38) made an excellent study of an outbreak of salmonellosis which he traced to shell eggs. *Salmonella montevideo* was isolated from two cases of shell eggs obtained on a merchant vessel. Twenty-eight individuals in a crew of 70 were affected after the consumption of contaminated egg salad. At attempt was made to trace the eggs back to the farm from which they had originated, but the work had not been completed at the time of the report.

All *Salmonella* cultures ferment dextrose, usually with gas production, but not lactose or saccharose. They do not liquefy gelatin, clot milk, or produce indole. *Salmonella typhi-murium*, *S. enteritidis*, and *S. panama* ferment arabinose and trehalose with acid and gas, whereas *S. cholerae-suis* does not ferment these substances. The last-named is the only one of the four which fails to produce hydrogen sulphide. All four produce acid in tartrate medium (39)—a fact which characterizes strains of animal origin.

The differentiation of a particular *Salmonella* is made by means of the agglutination test. This serological identification is made by antigenic analysis of somatic (O) and flagellar (H) antigens. By means of this test an antigenic formula has been given each of more than 150 species of *Salmonella*. In the formulas for antigenic structure in the Kauffmann (40) and White (41) schema, the Roman numerals refer to the somatic (O) (cell body) antigen which divides the *Salmonella* strains into groups. The small letters and numbers refer to the flagellar (H) antigens. For example, *S. typhi-murium* or *aertrycke* has the formula [I], IV, [V], XII:i:1, 2, 3, and *S. enteritidis* has [I], IX, XII:g, m:—. An International *Salmonella* Center has been established at the State Serum Institute, Copenhagen, which is financed by the Commonwealth Fund (42). This center will supply gratis to national *Salmonella* centers in the various countries some of the sera and cultures necessary for serological diagnosis and is prepared to examine doubtful cultures without charge. In the United States, a National *Salmonella* Center is located at the Communicable Disease Center Laboratories of the United States Public Health Service, at Lawson, Georgia, under the direction of Dr. P. R. Edwards. Dr. Edwards is a member of the International Committee. For a complete discussion of the serological identification test, the reader is referred to Kauffmann's book (40) or Circular 54, Kentucky Agricultural Experiment Station (43).

The evaluation of the significance of many of the species or types of *Salmonella* must await further investigation. The problems as to how fixed the antigenic components are and as to whether they may change and under what circumstances they may be altered are now under investigation in several laboratories. Perhaps the large number of types may be reduced, or simplified groupings be made when more of this basic knowledge is discovered. The H antigens of *Salmonella* can be markedly changed by cultivating the organisms in serum containing agglutinins for the flagellar components (40). P. R. Edwards and A. B. Moran (44) reported the occurrence in nature of H antigens apparently identical with those obtained by cultivation of *Salmonella* in agglutinating serum. Subsequently, D. W. Bruner and P. R. Edwards (45) were successful in inducing changes in the O antigens of *Salmonella* by following a method in which absorbed O antiserums were used in high concentration. Thus *S. anatum* was changed to a form indistinguishable from *S. newington,* and *S. meleagridis* was changed to a form indistinguishable from *S. cambridge.*

Salmonella aertrycke is now referred to as *S. typhi-murium* Castellani and Chalmers, 1919, since Loeffler in 1890 isolated it from a mouse epizootic, but it is known by many other names, such as *Bacillus psittacosis* Nocard, 1893; *Bacillus kaensche* Breslau bacillus von Kaensche, 1896; *B. aertrycke* De Nobele, 1899, 1901; *B. pestiscaviae* Wherry, 1908; *B. paratyphosis* B. mutton type, von Schütze, 1920; Group VII von Hecht-Johansen, 1923; *S. aertrycke* Ibrahim and Schütze, 1928, Warren and Scott, 1929–30, and Bruce White, 1929–30. In the German literature it has been called *Mäusetyphus-Bacillus* and *Bact. enteritidis* Breslau. The antigenic structure (40) is [I], IV, [V], XII:i:1, 2, 3.

Salmonella typhi-murium has been found in more food-poisoning outbreaks than has any other species of *Salmonella.* The many names for it are sufficient evidence of the confusion existing in attempts by bacteriologists to classify it. Epizootics among rabbits and guinea pigs have been frequently traced to *S. typhimurium.* It has been found to cause extensive outbreaks of dysentery and diarrheal disease among sheep and calves. It was thought by Nocard to have been the cause of psittacosis, since it was isolated from sick parrots; but it is now well recognized that psittacosis is caused by a filtrable virus.

Salmonella enteritidis Castellani and Chalmers, 1919, was isolated in 1888 by Gärtner in a meat-poisoning epidemic in Frankenhausen. Gärtner referred to it by the name *B. enteritidis*. Another synonym is Typhus Gärtner Jena, Kauffmann, 1930, 1931. The antigenic structure (40) is [I], IX, XII:g, m:—. Three varieties have been distinguished as based upon biochemical differences.

Salmonella enteritidis is found less frequently in food-poisoning outbreaks than is *S. typhi-murium*, but it causes the same symptoms clinically. It is the cause of epizootics among many species of animals. Calves are susceptible to infection with this organism, and rats and mice are found naturally infected with either or both *S. enteritidis* and *S. typhi-murium*.

Salmonella newport was first isolated from man in England (46). Since that time it has been found in numerous animal species (30), including fowls, ruminants, swine, carnivores, and rodents as well as man. It has the antigenic formula VI, VIII:e, h:1, 2, 3 . . . .

Salmonella oranienburg was first isolated from the feces of a healthy child in Germany. It has been found in fowl and in man (47), and there is considerable evidence supporting its pathogenicity for man. It has the following antigenic formula: VI, VII:m, t:—.

Salmonella montevideo was first reported by E. Hormaeche and C. A. Peluffo (48) in Uruguay from a monkey, from swine, and from children. It has a wide geographic distribution and has been found in fowl, swine, carnivores, and man (30). It has the antigenic formula VI, VII:g, m, s:—.

Salmonella cholerae-suis was first isolated and carefully studied by Salmon and Smith (7) in 1885. They spoke of it as the bacillus of hog cholera, but hog cholera is now recognized as a virus disease. Other synonyms are *B. cholerae-suis* Smith, 1893, 1894; *B. cholerae-suum* Migula, 1895 (Engler and Prantl, 1895); *B. suipestifer* Kruse, 1896 (Flugge, 1896); *B. salmoni* Chester, 1901. The antigenic structure (40) is VI, VII:c:1, 5.

Salmonella cholerae-suis has on only rare occasions been found to cause serious human infections (6). The natural and only host seems to be the pig.

Salmonella meleagridis was first described by D. W. Bruner and P. R. Edwards (49) after isolation from turkey poults in Minnesota. They (30) report it in fowl, swine, and man. It has a wide geographic distribution. The antigenic formula is III, X, XXVI: e, h:l, w.

Salmonella anatum was first isolated from ducklings in a case of mixed infection with *S. typhi-murium* and *S. newington.* It has been found in many parts of the world. It has the following antigenic formula: III, X, XXVI:e, h:1, 6 . . . .

Salmonella panama was isolated by Jordan (50) from an outbreak characterized by symptoms of dizziness, headache, and nausea (rarely vomiting), followed later by diarrhea and fever in about forty men belonging to two batteries of American soldiers in the Panama Canal Zone. The suspected food was meat hash served in the morning. The illnesses began to develop in the evening, but two of the men did not feel ill until the following morning. Nothing is known of the natural habitat of *S. panama.* Since Jordan's report, four cases (51) have been described in infants, of which two were fatal. The antigenic structure (40) of *S. panama* is I, IX, XII:l, v:1, 5 . . . .

Many more species or types of *Salmonella* than the few listed have been implicated in gastrointestinal disturbances in man. As pointed out in the beginning of the chapter, the pathogenicity for different individuals varies greatly, even with well-recognized types. There seems to be similar variability in pathogenicity for animals. For example (52), *S. aberdeen*, *S. give*, *S. california*, *S. worthington*, *S. oranienburg*, *S. paratyphi* B, *S. new brunswick*, *S. urbana*, *S. hvittingfoss*, *S. muenchen*, and *S. pullorum* were isolated from the intestinal contents or feces of chickens, and subcultures in veal-infusion broth containing 1 per cent starch were fed in 50-ml. amounts to *Macaca mulatta* monkeys. None of the monkeys developed any symptoms or signs of illness. Furthermore, viscera of swine (53), mostly cecum and colon from 16 different field cases of nondysentery enteritis, were fed to 27 cholera-vaccinated shotes without causing any significant effect. Living *S. cholerae-*

suis was present in at least 8 of the viscera which were fed to cholera-vaccinated pigs with negative results.

E. Hormaeche, C. A. Peluffo, and P. L. Aleppo (54) call attention to the greater susceptibility of young children to *Salmonella* infections. They (55) fed 5 human volunteers from two to four billion organisms of a strain of *S. typhi-murium*, isolated from a fatal case in a six-month-old child, and produced mild to fairly severe reactions of not more than 3 days' duration.

Obviously, many of the well-recognized types or species may cause gastrointestinal disturbances in man, and much more critical study is needed before the pathogenicity for man of some of the less common species or types can be properly evaluated. *Salmonella* are inhabitants of the intestinal tract of man and animals, and several species have been isolated from the stools of healthy man and animals. It is important that other causative agents be excluded in cases of gastrointestinal disturbances in evaluating the significance of a *Salmonella* type found in the stools. Recently in this connection *S. pullorum* (56) was implicated in an outbreak of gastroenteritis involving a known total of 423 persons, of which 172 were hospitalized. *Salmonella pullorum* was isolated from the stools of 11.7 per cent of the hospitalized patients, and it was believed to have been the probable cause of the infections. As pointed out elsewhere (57), no food items were available for bacteriological examination. Furthermore, the investigation was made after the peak of the epidemic and during the convalescence of patients. No report was made of a search for other agents. Another report (58) has appeared in which *S. pullorum* was isolated from the stools of a patient at the onset of diarrhea. Since man has been eating raw or incompletely cooked eggs for centuries, it appears strange that infections with *S. pullorum* should not have been discovered before the present reports.

In addition to the *Salmonella* group, recent attention has been given the Arizona group of paracolon bacteria as pathogens of animals and probably man (59). This group has been defined as motile, coliform bacteria which produce abundant H_2S but fail to form indol; are methyl-red positive and Voges-Proskauer negative; produce acid and gas from glucose broth; do not utilize *d*-tartrate or ferment sucrose, dulcitol, or salicin; ferment lactose with varying avidity and liquefy gelatin. This group was first studied by M. E. Caldwell and D. L. Ryerson (60) in strains isolated from horned lizards, Gila monsters, and chuckawalla. The strains were first classified as *S. arizona* with the formula XXXIII:z_4, z_{23}, z_{26} . . . but are now considered paracolon bacteria. E. Seligmann, I. Saphra, and M. Wassermann (61) found an identical culture in the feces of a woman affected with fever, vomiting, and diarrhea. Edwards (59) studied a culture from an infant affected with colitis.

As study of the pathogenicity of the enteric group of bacilli progresses, it is obvious that they form a gradient series of microörganisms with the more highly pathogenic typhoid and dysentery group at one extreme, grading down through the *Salmonella* and paracolon groups to the less pathogenic colon aerogenes group at the other extreme.

The detailed serological procedure for the identification of species is likely to lead to confusion unless carefully supervised. Simplified routine typing methods have been developed for use in diagnostic laboratories (62).

IS *Salmonella* FOOD POISONING AN INFECTION? (63, 64)

Some years ago, when the *Salmonella* group was the principal one considered by bacteriologists in food outbreaks, actual search for the living organisms in incriminated foods often ended negatively. Therefore, investigators became interested in the problem of whether or not thermostable toxins or toxic products were produced which would account for the outbreaks. Savage and White (16, 65) found that a substance is produced by the bacteria which is definitely toxic to laboratory animals if injected intravenously or intraperitoneally. Others (66, 67, 68) corroborated this work. Several investigators (69, 70, 71) found that boiled *Salmonella* cultures, when fed to mice, caused death in a large proportion of them but might or might not cause death when fed to other animals (17). G. Elkeles (72) has reviewed the literature on this subject.

In the reports dealing with the feeding of heat-killed *Salmonella* cultures to animals, the majority of positive results has been obtained with mice. Because the mouse reacts in this way, however, is no reason to assume that other species, including man, react similarly. As a matter of fact, the experimental evidence demonstrates that susceptibility in the mouse, as tested by the oral route, differs from that of other animals. For example, Bahr and Dyssegaard (67) fed rhesus monkeys as much as 950 ml. (1 qt.) of heat-killed cultures over a period of about 4 days. The initial feeding was usually 250 ml., followed by 2 other feedings of 350 ml. during the course of the next 3 days. No symptoms were observed, but appreciable agglutinins were demonstrated in their blood serum. In experiments carried out in our laboratory, heat-killed cultures of *S. typhi-murium* fed to 4 rhesus monkeys failed (with 1 possible exception) to produce gastrointestinal symptoms characteristic of paratyphoid intoxication. On the other hand, living cultures of *S. typhi-murium* and *S. enteritidis* fed to 11 rhesus monkeys caused watery diarrhea and general malaise, with loss of appetite in some cases (73). Recovery was prompt and apparently complete; the specific bacillus was not found in the blood stream. Monkeys fed with equivalent heat-killed portions of the same suspension showed no symptoms. Likewise, living cells of *Proteus* and *Bact. coli* failed to produce any noticeable effect.

The crucial test as to whether or not heat-stable products of the food-poisoning types of *Salmonella* may cause illness in man rests upon feeding such material to man. This has been done with negative results (74). Heat-killed, dextrose-broth, beef-heart cultures and filtrates of 5 strains of *S. typhi-murium* and 4 strains of *S. enteritidis*, when fed in amounts of 20–340 ml. (0.66–11 oz.) to 24 adults on an empty stomach, failed to produce any symptoms, although the same materials produce symptoms and death when injected intravenously into rabbits in 0.5–2-ml. amounts. No agglutinins for homologous strains were present in the serums of these subjects 10 days after feeding. One objection which may be raised to these experiments is the fact that freshly isolated strains of *Salmonella* were not used. The answer to this objection may be found in the work of Verder and Sutton (14), who demonstrated that a living culture of *S. enteritidis* which had been under cultivation on artificial medium for 2 years produced typical symptoms of food poisoning when eaten by man in custards. Moreover, heated suspensions, as well as unheated filtrates of a culture isolated from the stools of this person and grown in custard, were fed to 7 human volunteers, none of whom became ill. Similarly, heated and filtered cultures of the same *enteritidis* strain produced no symptoms of food poisoning in monkeys, whereas living organisms, when fed, made the animals ill. From this experiment it is evident that filtrates or heat-stable products of a freshly isolated culture after passage through man are harmless when fed to man.

Epidemiological evidence appears to corroborate the experimental evidence that living organisms are essential in outbreaks of food-borne infection caused by *Salmonella*. Hammerschmidt (18) calls attention to the fact that in the course of an epidemic only those people who ate uncooked or semicooked meat became ill, whereas those who ate cooked meat did not become ill. He also observed that people who did not eat the incriminated food were made ill by contact with persons actually ill. The evidence at the present time is overwhelmingly in favor of the view that *Salmonella* food poisoning is actually an infection and that toxins or toxic substances play no role.

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CHAPTER VII

Alpha-Type Streptococci (Streptococcus faecalis) in Relation to Food Poisoning

ALPHA-TYPE streptococci grow readily on the surface of blood agar plates, and about the tiny pin-point colonies a greenish zone develops, owing to the chemical change in the hemoglobin. This ability to produce a greenish zone of hemolysis about the colonies is the basis for the classification (1) of the "alpha (α) type." Many workers (2, 3, 4) have considered this type of streptococcus responsible for food-poisoning outbreaks when it predominated in suspected foods, although the incriminated food when fed to animals caused no illness. B. A. Linden, W. R. Turner, and C. Thom (5), however, actually found symptoms of food poisoning in cats after feeding them living cultures of streptococci isolated during an outbreak (5). Unfortunately, the reactions of experimental animals may not be the same as those of man. This streptococcus strain was saved and was studied several years later by J. M. Sherman, K. L. Smiley, and C. F. Niven, Jr. (6), and was found to be *Streptococcus faecalis*, belonging to the Lancefield serological group D.

In our laboratory, four outbreaks in which α-type streptococci predominated in the incriminated food have been studied bacteriologically. In two of these, involving canned Vienna sausage (7) and beef croquettes (8), cultures of the organisms and filtrates have been studied in human volunteers. The other two involved cocoanut-cream pie (8) and turkey dressing (not previously reported). The first of these outbreaks involved 75 of 182 boys who had eaten a Sunday-night meal at which Vienna sausage had been served. Other foods and water could be ruled out on epidemiological grounds. The sausages were taken from 6 large (No. 10) cans and were served without heating. There was no abnormal appearance, odor, or taste. Since less than half the boys became ill, and only 1 of 4 unopened cans contained the organisms, it is probable that only 1 or 2 cans had been contaminated. The symptoms included nausea, vomiting, colic-like pains, and diarrhea. Most of the boys began to be ill within 4–5 hours, but a few did not begin to exhibit symptoms until 12 hours after supper. All recovered within 24 hours after the onset of symptoms.

Three sausages left over from the supper were examined bacteriologically and found to contain large numbers of slowly growing, pleomorphic, α-type streptococci. These organisms in veal-infusion broth cultures were readily killed when heated to 60° C. for 1 hour or 85° C. for 15 minutes. No *Salmonella* were found. Four unopened cans of sausage, with the same code-marking, were later examined. One of these contained viable organisms essentially identical with those isolated from the sausages left from supper. The unopened can in which the contaminated sausages were found showed defective seams through which the streptococci may have entered. When cultures from the unopened can had been incubated for 4 days, but before growth was evident, a young adult laboratory worker ate half a sausage from each of the 4 cans. Five hours later she developed nausea, which increased for 24 hours, with 2 periods of vomiting accompanied by epigastric pain and exhaustion. A second volunteer drank 40 ml. of a 5-day beef-heart culture and became ill 5 hours later. Belching was followed in 24 hours by unsuccessful attempts at vomiting, intermittent epigastric pain, tenesmus, cold perspiration, a temperature of 99.2° F., and marked exhaustion. A third volunteer drank 50 ml. of a bacteria-free filtrate prepared from a similar culture and suffered no ill effects. Mice, guinea pigs, rabbits, and monkeys (*Macaca mulatta*) were fed cultures of these streptococci but showed no symptoms. Three young cats which were fed 48-hour milk cultures had soft stools the following day but otherwise appeared to be unaffected.

The outbreak involving croquettes (fried minced-meat balls with rice and egg) occurred in a group of about 250 men between seventeen and twenty-five years of age. The noon meal was found on epidemiological evidence to have been responsible. Samples of all remaining foods were sent to the laboratory 2 days later. Both epidemiological and laboratory evidence showed that beef croquettes were definitely implicated. These croquettes were prepared from the meats remaining from the meal of the preceding evening, consisting of beef stew, round of beef, and Swedish meat balls. The meats were assembled in a huge container and were left covered at room temperature until the following morning, when they were placed in the refrigerator until time to prepare the croquettes. They were then chopped together mechanically and mixed with bread crumbs and gravy made 2 days previously. The croquettes were fried in deep fat at about 110° C. for 10 minutes while the meal was being served. They apparently tasted all right, for several individuals had a second helping. Conditions in the kitchen were sanitary, and no rat or vermin poison had recently been used. The kitchen employees had been given health examinations routinely. The cook had a head cold on the day that the croquettes were served.

By the questionnaire method, information was secured from 208 of the young men. Of this number, 117 were made ill, and 91 were not affected. Sixty-three of those not ill ate croquettes. This fact may have been due to a relatively high resistance of the young men or a variation in the time of frying the croquettes. Among the 117 who were ill, 98 reported that they had eaten the croquettes. Since the questionnaire was circulated nearly a week after the outbreak, some discrepancies in reporting were to be expected. Diarrhea, the outstanding symptom, was reported in 110 cases, whereas only 3 reported vomiting, 15 nausea, and 55 abdominal cramps. The incubation period was from 6 to 18 hours in 104 of the boys and was generally about 12 hours. In 3 of the boys, however, it was 3 hours or less. The symptoms subsided in from 6 to 24 hours in all except 4 cases; in these 4 they persisted for over 3 days. It is readily seen that this outbreak was unusually mild and consisted essentially in the development of diarrhea after an incubation period of several hours.

No *Salmonella* were found in specimens of the food, but numerous α-type streptococci were present on the blood agar plates. Five days after the outbreak, a weighed sample of the refrigerated croquettes was ground with sterile sand, taken up in saline, and plated in decimal dilutions in blood agar. After the culture had grown for 24 hours, a plate count of 280 million green-producing streptococci was found per gram. This was not an exact count, because the streptococci were clumped, and the count was not made immediately after the outbreak. Seven volunteers between the ages of twenty-two and thirty years, who were not subject to spontaneous gastrointestinal disturbances, drank milk to which had been added varying amounts up to 20 ml. of the filtrate of the culture grown in veal-infusion broth. None was made ill. Five of the 7, however, felt abdominal distress after taking broth cultures containing living streptococci one or more times.

From the two foregoing instances, it will be seen that, though α-type streptococci may give rise to symptoms of food poisoning in man, culture filtrates of these organisms do not.

The outbreak caused by turkey dressing occurred at a banquet at which 393 people were served and three-quarters of them were made ill. Fifteen or 16 fresh turkeys were stuffed on one evening, placed in the refrigerator overnight, roasted from 8:00 A.M. to 1:30 or 2:00 P.M. on the next day, left in open pans to cool, carved at about 4:30 P.M., and placed on top of the range in open pans until they were served 5 or 6 hours later. The meal was eaten between 8:30 and 9:00 P.M. in the evening, and illnesses began to occur at about 4:00 A.M. the next morning and continued on until noon. The incubation period, therefore, was from 7 to 15 hours. The symptoms consisted generally of abdominal cramping and diarrhea, with some nausea, vomiting, and prostration. Blood agar plates streaked with a suspension of either the turkey or the turkey dressing and incubated for 24 hours at 37° C. contained numerous colonies of α-type streptococci in almost pure culture. A study of this strain by Buchbinder *et al.* (9) showed it to be *S. faecalis*. No *Salmonella* were found on plates of eosin methylene blue agar streaked with the specimen.

Streptococcus faecalis has been found in samples of dried egg which are known to be toxic to man when eaten (10), as well as in many samples of dried egg not known to be toxic. A 10 per cent suspension of dried egg in quarter-strength Ringer solution was heated in a 70° C. water bath for 1 hour for three periods, each followed by a 3–6-hour interval of incubation at 37° C. After the last heating period, 50-ml. quantities were inoculated with $\frac{1}{50}$–$\frac{1}{25}$ ml. of a 1½–2½-hour actively growing culture of *S. faecalis*. The egg cultures were incubated for 24–48 hours, with final colony counts of 100,000,000–750,000,000 organisms per milliliter. Ten-ml. portions heated in a 70° C. water bath for 45 minutes were fed to 5 human volunteers without producing ill effects.

Buchbinder and his co-workers (9) reported bacteriological studies and other pertinent data in four outbreaks of food poisoning, in each of which enterococci were the predominant organisms isolated from the suspected foods (canned evaporated milk, charlotte russe, roast beef, and ham bologna).

In the outbreak involving canned evaporated milk, the cans were from a lot stored or handled under conditions causing spoilage, since many cans were rusted or were leakers, springers, or swells. The milk had been canned two years previously. This outbreak, attributed to evaporated milk, occurred in a children's institution. There were 74 individuals out of 161 ill within 2–7 hours after a meal, with symptoms characterized by abdominal cramps, nausea, and vomiting. Diarrhea was reported in only two cases, and all were symptom-free within 6 hours after onset. Mashed potatoes and pasteurized milk were the only foods common to those who were ill. Unfortunately, none of the implicated foods was examined, and the evidence was circumstantial. Heat-stable staphylococcus enterotoxin in the absence of viable staphylococci is not excluded as a cause of this outbreak.

The outbreak involving charlotte russe (sponge cake topped with a simulated whipped cream) concerned three members of a family who became ill within 5½–12 hours after a meal. Symptoms consisted of abdominal cramps, vomiting, and diarrhea. The charlotte russe was the only food eaten in common. Specimens of the ingredients used in making this desert were obtained from the bakery and consisted of sponge cake, gelatin, skimmed condensed milk, and whipped cream. The sponge cake contained only about 800 bacteria per gram, which were almost entirely coliform organisms. The gelatin contained 3×10^5 organisms per gram, although prior to its use in the pastry it had been heated in a double boiler for 15–18 hours. A bacterial count of 54,000 organisms per milliliter was present in the skimmed condensed milk, mostly enterococci. The whipped cream yielded a total of 1×10^7 organisms per gram, which were largely *S. faecalis*.

The outbreak implicating barbecued beef involved a group of people who had attended a wedding anniversary dinner. The incubation period was generally 10–12 hours, the extremes being 3 and 15 hours. Symptoms were mild, a few individuals were nauseated and vomited, whereas the majority experienced only abdominal cramping and diarrhea. Seventy-four of 87 persons interviewed had become ill. The meat had been roasted over hickory logs on the evening before it was consumed and had been left unrefrigerated in the roasting-room of the delicatessen from 10:30 P.M. until 11:00 A.M. the following morning. About 50 pounds of it were then sliced and taken to a synagogue, where it remained unrefrigerated until 3:00 P.M. At that time the meat was placed in pans and heated in a slow oven for 1 hour. It was consumed at 4:00–5:00 P.M. Samples of the meat and gravy, made up of turkey and chicken drippings, served with the meat were taken from refrigerators, where the leftover food had been placed following the dinner. A count of 4.5×10^6 organisms per gram, with enterococci predominating, was found for the meat and 1×10^5 to 2.4×10^5 per milliliter for the gravy.

The ham bologna involved in the fourth outbreak was part of one shipment distributed by a large chain retail grocery company to a number of its stores in the New York City metropolitan area. The company had been notified of additional outbreaks outside the city and had recalled all the meat. Nine persons in 3 families ate the ham, and symptoms began in 3–6 hours and lasted from 3 to 48 hours. They consisted of vomiting, abdominal cramps, and diarrhea. A count of 3.5×10^6 organisms per gram, mostly *S. faecalis*, was found.

Experimental studies have been carried out in which the α-type streptococcus (*S. faecalis* from the outbreak implicating turkey dressing previously mentioned) was inoculated into various types of canned goods (11). The organism was demonstrated for at least 30 days at 37° C. in canned foods of low-acid content (corn, peas), semiacid content (asparagus, spinach, string beans), a highly acid product (tomato juice), and in sea-food products (shrimp and salmon). Organisms were demonstrable in all cases for 60 days at 22° and at 37° C. in most instances. Growth did not occur in canned peaches, sugar-preserved (a highly acid product); and viable organisms were not cultured after 2 days. Although α-type streptococci are killed by the time and temperature used in processing canned foods, the ability of these organisms to grow and survive in such products is of practical importance to the problem of food poisoning. Contamination of the contents of a can with these organisms may occur after the can is opened, after which storage at temperatures supporting growth might lead to the development of large numbers of organisms capable of giving rise to gastrointestinal symptoms on ingestion. The physiological characteristics (12) of *S. faecalis* are such that it may well find conditions in the opened can of food suitable for multiplication. For example, *S. faecalis* is characterized by its ability to grow fairly rapidly over a wide temperature range (10°–45° C.) or to grow in salt concentrations of 6.5 per cent and higher and, finally, by its relative heat resistance, surviving the time and temperature of pasteurization.

It is evident, therefore, that the incubation period from the time of eating food contaminated with α-type streptococci to the onset of illness is longer than that of staphylococcus food-poisoning outbreaks and varies from 2 to 18 hours. In children the incubation period may be shorter than in adults. Symptoms of this type of food poisoning are usually much milder than in staphylococcus food poisoning, and the onset of illness is spread out over a longer period of time. This longer period seems to occur when living agents are involved, as in α-type streptococcus and *Salmonella* infections of the food-poisoning type, whereas a shorter period occurs if enterotoxin is responsible (cf. Table 13). As stated previously, the longer incubation period may represent the period required for multiplication of the organisms in the intestinal tract before the infection is manifested.

Sherman and his co-workers (13) are responsible for recognizing *S. faecalis* as the species causing this type of food poisoning. They found that a total of 6 strains of nonhemolytic streptococci isolated by others from an equal number of outbreaks were *S. faecalis*. When studied carefully, no other species of α-type streptococcus has been encountered. Perhaps only a small number of *S. faecalis* strains possess this property of causing gastrointestinal symptoms. Future studies will have to settle this problem. Certainly, the species is a natural inhabitant of the intestinal tract of man and animals and, as a consequence, is probably ingested in man's food and drink. In all outbreaks enormous numbers of *S. faecalis* are ingested in the implicated foods, and dosage undoubtedly plays a role in the initiation of illness.

TABLE 13

CHARACTERISTICS OF FOOD POISONING CAUSED BY BACTERIA OR THEIR PRODUCTS

Disease	Specific Agent	Intoxication	Infection	Symptoms	Onset of Symptoms after Eating
Botulism	*Clostridium botulinum* which produces toxin	+	−	Difficulty in swallowing, double vision (diplopia), difficulty in speech (aphonia), difficulty in respiration, followed by death from paralysis of muscles of respiration	2 hours to 8 days; average 1–2 days
Staphylococcus food poisoning	Staphylococci which produce enterotoxin	+	−	Nausea, vomiting, diarrhea, and acute prostration; abdominal cramps	1–6 hours; average 2½–3 hours
Salmonella infection	*S. typhimurium* *S. enteritidis* *S. choleraesuis*	−	+	Abdominal pain, diarrhea, chills, fever, frequent vomiting, prostration	7–72 hours
Streptococcus food poisoning	α-type (*S. faecalis*)	−	+	Nausea, sometimes vomiting, colicky pains, and diarrhea	2–18 hours

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CHAPTER VIII

Significance of Other Bacteria in Food Poisoning

MANY microörganisms other than those previously referred to have been implicated in outbreaks of food poisoning, but the evidence incriminating them is not conclusive. An interesting example may be cited from studies of water-borne outbreaks of typhoid fever. When water is suddenly contaminated with sewage, cases of diarrhea, abdominal cramping, and nausea frequently occur within 12–48 hours. This relation was shown by E. O. Jordan and E. E. Irons (1) in an outbreak in Rockford, Illinois, in 1912 and is well summarized in a report on a subsequent outbreak occurring in another city. The authors make the following statement:

> Thus in the Rockford epidemic, due to a sudden and temporary pollution of the water supply by sewage, the probable number of cases of initial enteritis was over 10,000 in a population of 50,000 but careful search for cases of typhoid revealed only about 200 cases. Out of the total of 46 persons residing in one block, 44 drank sufficient water to produce gastro-intestinal disturbance, in some instances, mild with little or no diarrhea, in others, very severe with nausea, vomiting, purging and marked prostration, and yet only one person of the 44 suffered from typhoid fever later [2].

A similar example came to the author's attention a few years ago when there was a large fire in the Chicago Stock Yards. Much water was pumped from the mains at this time, and sewage gained entrance to the water of the district from a drainage ditch. After 8 hours or more, a number of individuals who drank from the hydrants in the area at that time suffered acute gastrointestinal upsets. Symptoms were mild and disappeared in a few days. Nevertheless, in due course of time, several cases of typhoid fever and amebic dysentery occurred among the populace drinking the contaminated water. The cause of the acute gastrointestinal disturbances which follow drinking sewage-polluted water after a few hours has not been established but may be a specific pathogenic bacterium.

Bacterium coli

Bacterium coli has many times been associated with outbreaks of food poisoning, but there is no definite proof that this organism is the responsible agent. H. Lodenkämper (3) has reviewed the literature and reported outbreaks of food poisoning which he believes are caused by *Bact. coli.* His evidence is based on the search for the causative organisms on Endo's medium and for toxins in broth. Tests for a toxin were made by oral and intraperitoneal injections into mice. This type of evidence is not conclusive, as has been previously discussed under *Salmonella.* Plating on Endo's medium is unsatisfactory for detecting staphylococci and streptococci, which, therefore, may be missed.

W. G. Savage makes the following statements in regard to the role which intestinal bacteria play in food-poisoning outbreaks:

> In the first place massive bacterial infection with intestinal bacteria is exceedingly common and foods such as milk, ice cream, brawn and sausages are habitually consumed containing thousands of bacteria per cubic centimeter or gramme, many of which are of direct intestinal origin. Food poisoning outbreaks should be of repeated and constant occurrence instead of being comparatively infrequent [4].

In another place he writes:

> We have finally the impressive fact that the more carefully and fully individual outbreaks are studied and bacteriologically investigated the greater the number found to be associated with infection with some definite bacillus, and it is a reasonable assumption that the residuum of outbreaks unassociated in this way would be comparatively small if all were fully investigated, bacteriologically and chemically, and especially if material was available for examination early in the course of the outbreak.

Proteus

Proteus vulgaris has frequently been associated with food poisoning. An outbreak was reported in Bristol, England (5), after brawn had been eaten which was supposed to be contaminated with toxin produced by organisms of the *Proteus* group. The evidence was based on the intraperitoneal injection of kittens with filtrates prepared from cultures grown on semisolid agar in 10 per cent carbon dioxide overnight. Evidence of this type is valueless, since it has not been established that the intestinal tract is permeable to the toxic agent which is demonstrated by parenteral injection.

In this country an outbreak of food poisoning involving 34 people was traced to smoked fish (6). No specific food-poisoning organisms were isolated by bacteriological examination, but several samples of the fish had high bacterial counts, with *Proteus* predominating. No proof was brought forth, however, to show that *Proteus* was responsible for the food poisoning.

In an outbreak of food poisoning traced to sausage (7), some 2,000 soldiers were affected. The illnesses were characterized by vomiting, diarrhea, and exhaustion. The symptoms appeared in most cases in 2–3 hours after eating sausage. A low-grade fever occurred in only the severe cases, which promptly subsided following treatment. Two hundred of the soldiers were hospitalized. Those who did not eat sausage were not ill nor were those who had eaten from the same shipment of sausage 2 days earlier. A bacteriological examination of sausage revealed cocci and *P. vulgaris*. No *Salmonella* were found. The symptoms were those of staphylococcus food poisoning, although *Proteus* was listed as the causative agent, since it occurred before staphylococcus food poisoning was generally recognized.

Proteus mirabilis was thought to have a probable etiologic relationship in an outbreak of food poisoning occurring in the Navy and traced to baked ham (8). In this outbreak 29 cases were reported, and 19 were hospitalized. Symptoms were severe and consisted of nausea, vomiting, and diarrhea. The onset of symptoms averaged 3 hours after eating. Of 19 hospitalized patients, 3 passed blood, 16 had abdominal cramps, and 3 had fevers with a maximum of 101° F. Sixteen had normal or subnormal temperatures. *Proteus mirabilis* was isolated from suspected ham and from 9 of 19 patients studied. A bacteriological examination of the ham revealed 62,000 organisms per gram, and, of this number, 12,000 per gram were staphylococci. Staphylococcus food poisoning was considered unlikely because of the low count of these organisms. No record was given as to whether the ham had stood at a warm temperature for a number of hours before cooking. Under these conditions the heat-stable staphylococcus enterotoxin would still be present after cooking, although the organisms would be killed.

Proteus organisms are common inhabitants of the intestinal tract of man and animals. The numbers of these organisms may be, but usually are not, markedly increased when intestinal function is impaired. In a study of the isolated colon of a patient (9) with an end-ileostomy, *Proteus* organisms were frequently encountered at times when the colon was in a healing phase. During one period of 112 days, during which oxygen insufflation of the colon was carried out, 43 bacteriological examinations were made, and it was observed that *Proteus* colonies appeared in large numbers only during short periods of improvement of the colon. It has been the author's experience that, in patients with chronic ulcerative colitis when the colon was acutely involved in exacerbations of the disease, *Proteus* was usually absent, whereas, during periods of remission, *Proteus* was often found in large numbers.

Proteus organisms are commonly found in large numbers in the feces of *Macaca mulatta*. They were found in the isolated colon of one of these animals in large numbers (10). However, when 10 ml. of defibrinated sterile sheep blood was injected daily into the isolated colon, *Proteus* colonies were absent after 2 weeks and did not reappear until 4½ weeks later, although daily blood injections were given over a period of 55 days.

An outbreak (11) of food poisoning involving 180 persons was traced to the meat from a hog which had showed evidence of septicemia at the time of slaughter. *Proteus* and colon bacilli were found in large numbers in the meat, but, by using selective media, *Salmonella* organisms were recovered. Since *Salmonella* organisms were found in the stools of patients and specific agglutinins were found in the serum, *Proteus* and colon bacilli were excluded as possible causative agents.

Savage (4) has reviewed the literature on 9 outbreaks of food poisoning attributed to *Proteus* and concludes: "From this summary of outbreaks it is evident that for none of them was it established that *B. proteus* was etiologically concerned."

Proteus is commonly encountered in the intestinal tract of man and animals who are not suffering from food poisoning. Its common occurrence when food poisoning is not concerned must, therefore, be explained if etiological significance is to be given it in food poisoning.

CLOACAE-AEROGENES GROUP

Early in 1930 an outbreak of gastroenteritis attributed to cream-filled pastry occurred in an area of 20 square miles in New York and New Jersey (12). The symptoms were said to have been manifested in every case within a few hours after the eating of either cream puffs or chocolate éclairs manufactured by a wholesale bakery. About 125 cases developed on 2 successive days. *Staphylococcus aureus* was found in large numbers in the cream filling. It was excluded by the authors because animal-inoculation tests were negative. Thus, 5 ml. of a 48-hour culture grown in medium containing ingredients similar to those used in the pastry filling failed to cause symptoms when fed to a monkey. A guinea pig and a mouse were inoculated intraperitoneally with 2 ml. and 1 ml., respectively, of a filtrate from it. Two rabbits were inoculated intravenously with 3 ml. either of this filtrate or of a filtrate from a broth culture. None of the animals showed any evidence of illness. An organism belonging to the cloacae-aerogenes group was thought to be responsible, since it was isolated from 5 fecal specimens and from the filling of 5 cream puffs or éclairs. The growth products of this organism proved toxic for certain animals. This outbreak with its short incubation period was, however, in all probability due to staphylococci, since it is obvious that the above tests did not exclude staphylococcus food poisoning. As shown previously, the monkey is extremely resistant to the enterotoxin as compared to man; and mice, guinea pigs, and rabbits are unaffected by the parenteral injection of enterotoxin. Similarly, such animals are unaffected by the injection of boiled filtrates. When filtrates are boiled, the other toxins are greatly weakened or destroyed, and the enterotoxin is not appreciably affected. One cannot emphasize too strongly that filtrates of cultures of the common gram-negative intestinal bacteria may be toxic when injected into animals and yet be without effect when fed to man (13).

PARACOLON ORGANISMS

This is a large group of heterogeneous coli-like organisms which have certain physiological characteristics unlike typical *Escherichia*. Taxonomically, some workers (14) have considered them intermediate between the typical coliforms and the salmonellas. In a small outbreak of gastroenteritis (15) a subgroup of these organisms was isolated from stool and rectal-swab specimens from 17 of 52 patients. The implicated food was corn pudding, and none was available for laboratory study. The incubation period averaged 12 hours, and the symptoms in the 52 cases were characterized by diarrhea (92.3 per cent), abdominal cramps (75 per cent), nausea (50 per cent), and vomiting (11.5 per cent). Recovery was complete in 12 hours. No tests were made for α-type streptococci, since none of the food in question was available.

Clostridium welchii

Clostridium welchii has been implicated in an outbreak in babies and children (16) where milk was heavily contaminated with the organism. The illness was characterized by fluid stools with an offensive odor and by fever. The abdomen was acutely distended by gas in the intestinal tract, and pus was present in the stools. Since the diarrhea and abdominal distention promptly subsided and the stools became normal when a noncontaminated acid milk was fed, it would suggest that the symptoms were due to an active fermentation process in the intestinal tract with mechanical distention of the bowel, giving rise to nausea and hyperperistalsis.

In three food-poisoning outbreaks (17) in which *Cl. welchii* was implicated, at least 20 people were involved. The incriminated food was creamed-chicken croquettes from precooked chicken. These illnesses were characterized by nausea, vomiting (rare), intestinal cramps, and diarrhea, beginning 8–12 hours after the meal and continuing for 12 hours. Illness was mentioned in one human volunteer. The author suggests that *Cl. welchii* may at times produce potent enterotoxin for man, although proof for this contention is lacking

Clostridium welchii is present in soil, water, milk, dust, sewage, and the intestinal canal of man and animals (18). It has been shown to be present in commercial "bread starter" for salt-rising bread (19), as well as in the baked loaves. It is possible that *Cl. welchii* in large numbers in certain foods may ferment these foods after ingestion, thus producing gastrointestinal symptoms. However, in the majority of instances its importance in food poisoning is not great.

A vigilant search for new causative agents of food poisoning should be maintained, but caution should be exercised in multiplying the number of microbial agents on the basis of presumptive but inconclusive evidence (20). Year after year some of the same agents are reported in the literature without additional proof as to their etiological significance.

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CHAPTER IX

Infections To Be Differentiated from Food Poisoning

THERE are many infections which may simulate food poisoning, in that they may give rise to diarrhea, abdominal cramping, and nausea with or without vomiting and pyrexia. These infections must be ruled out in dealing with food poisoning. Since they are not associated with food poisoning per se, no detailed discussion will be given of them. As pointed out before, however, there is one outstanding difference. Food poisoning is generally of an explosive nature, whereas epidemics of infectious diseases may break out over a period of several days.

BACILLARY DYSENTERY

Upon two occasions the author has been called upon to investigate supposed outbreaks of food poisoning which were found to be bacillary dysentery. Although much has been written about this disease and many infectious agents have been described, little is known of the natural habitat of the organism. In areas in which typhoid fever and cholera are controlled, bacillary dysentery remains endemic. It is common where groups of people are together, as, for example, in prisons, asylums, armies, and among populations in summer-resort areas. In recent years better selective media have been devised for the isolation of dysentery bacilli from feces; but it is well known that during convalescence the organisms often disappear rapidly from the stools and cannot be detected with the most selective laboratory methods. The fact that bacillary dysentery is so common indicates that there are many undetected carriers. This contention is justified by findings in the monkey (*Macaca mulatta*), which is a natural host of the Flexner type of bacillary dysentery. Although dysentery bacilli may not be recovered from the stools of these monkeys by the best technical procedures, the animals spontaneously develop the disease (1, 2, 3) when placed on vitamin-deficient diets.

Shaughnessy and his co-workers (4), in testing the effectiveness of vaccines for bacillary dysentery, challenged human volunteers with freshly isolated strains of *Shigella paradysenteriae* (Flexner W).* They fed 100,000,000 living organisms in milk to 4 unvaccinated persons without producing diarrhea or fever. In a second group of 4 volunteers who received 1,000,000,000 living organisms of the same strain in milk, none developed temperature, and one patient passed four watery stools in the second day after he had drunk the culture. Positive stool cultures occurred in both groups within 48 hours. In a third group, 4 subjects were fed 10,000,000,000 living organisms of the same strain in milk. None showed elevated temperatures. A fourth group of 4 volunteers received 10,000,000,000 living organisms of the same strain in water.

Three of the 4 of this group were definitely ill, whereas the fourth experienced only slight cramps. The 3 who were ill experienced cramps within a few hours after the dysentery bacilli were swallowed. Two of the 3 had frequent watery stools. The third patient vomited and had severe cramps for as long as the tenth day after inoculation. In a fifth group of 4 subjects, 15,000,000,000 organisms of the same strain were fed in water, and none showed any signs or symptoms during the next 24 hours. So they were given 15,000,000,000 more on each of the next 2 days without effect. On the fourth day they were given 50,000,000,000 more, and only mild headache, anorexia, and slight abdominal cramps and an occasional soft stool resulted.

In addition to the above tests, other volunteers were fed human feces infected with the same strain of *Sh. paradysenteriae*, and other volunteers were fed more recently isolated strains. Of 30 volunteers who received no vaccine and who were fed living *Shigella* cultures, only 19 developed dysentery. The incubation period in the experimental disease was, with few exceptions, not longer than 24 hours. A strain of *Shigella* virulent for Swiss mice when injected intraperitoneally failed to produce clinical infection in man, while other strains of much lower virulence for mice produced severe dysentery in man. These experiments illustrate the variability of the susceptibility of man to infection, as well as the importance of the virulence of the organism in producing infection. Furthermore, the fallacy of attempting to evaluate the pathogenicity of these organisms for man on the basis of parenteral injections into other animal species is well illustrated. This experimental study with *Shigella* parallels closely some of the results obtained in similar studies with *Salmonella*.

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* Each volunteer received 1 drachm (4 ml.) of paregoric, 3½ hours after a meal, followed ½ hour later by 2 gm. of powdered sodium bicarbonate in 1 ounce of water. Five minutes later the dysentery bacilli were given, suspended in ½ pint of milk or, in later experiments, in a glass of water.

Bacillary dysentery may be spread by food contaminated with feces from carriers or from those actually suffering from the disease. In any outbreak, however, the cases appear over a period of days rather than within a short space of time. This difference, together with the recovery of the bacilli from stools by culture, is sufficient to differentiate the disease from food poisoning. It is recommended that cultures of stools be made in all cases in which bacillary dysentery is suspected.

INFECTIOUS HEPATITIS

A presumably food-borne outbreak of infectious hepatitis occurred in a fraternity house (5). In most cases the onset of the disease was insidious. In addition to malaise, general myalgia, chills, and fever, anorexia appeared early and, by the end of 4–7 days, had become severe. In many cases almost intractable vomiting occurred. Dark urine was noticed soon after the onset of the illness, and by the fourth or fifth day fever had begun to subside and jaundice followed. In this outbreak 24 cases occurred between May 26 and June 5, 1944. Of 101 members of the fraternity, 14 men lived in the house and ate all their meals in the house dining-room. Four cases developed in this group, constituting an attack rate of 28.5 per cent. Of 60 men eating all or part of their meals at the house, but not living there, were 20 cases, an attack rate of 33.3 per cent. Of the remaining 27 members eating no meals or only an occasional meal at the house, none was ill. No cases occurred in students other than members of the fraternity, even though the fraternity students had contact with other students. Spirochetal jaundice was excluded.

AMEBIC DYSENTERY

Amebic dysentery may occur in epidemics, as was demonstrated at the Century of Progress Fair in Chicago in 1933 (6). It generally occurs sporadically and may continue to occur over a long period of time. The Chicago epidemic was water-borne and was shown to arise in individuals staying at certain hotels in which the plumbing was faulty and a back siphonage of sewage occurred in the water lines. It may also be food-borne. Usually the disease is endemic and is spread by intimate personal contact. Its insidious and chronic nature differentiates it from food poisoning. The incubation period from the time of ingestion of the amebae to the onset of symptoms may vary from less than a week to, in a few cases, 4 months. The attacks vary from mild intermittent diarrhea to an acute fulminating type of bloody flux. Many of the people apparently acquiring the infection during the Chicago epidemic did not develop symptoms until some time after they returned to their homes in other parts of the country. *Endamoeba histolytica*, the causative agent of amebic dysentery, should always be excluded before making a diagnosis of food poisoning, especially if only a few cases are involved. Detailed methods have been devised for the laboratory diagnosis of this protozoan (7, 8). Its presence in stools should be interpreted with caution, however, as approximately 10 per cent of the population are infected, and the majority of these suffer no noticeable symptoms.

TRICHINOSIS

The early stages of this disease have been confused with food poisoning. Some years ago the author was consulted about some cases in which physicians were considering the diagnosis of botulism, since several of the affected people died. This outbreak occurred in an Italian family. Each member of the family developed acute diarrhea, and two of the children died. At necropsy, larvae of *Trichinella spiralis* were seen in abundance in the muscle. An inquiry into this outbreak revealed the source of the infection. The father of the family had brought home a dead hog which he had found on a dump and had made into sausage without cooking. Some of the sausage was found to contain numerous encysted larvae.

This disease is caused by a nematode, *T. spiralis*, which is usually obtained by man from insufficiently cooked, infected hog meat. A description of this infection may be found in textbooks of parasitology. When encysted larvae are eaten, the cysts are digested in the patient's stomach, whereupon the larvae excyst in the duodenum. They invade the duodenal and jejunal mucosa, giving rise to such symptoms as nausea, vomiting, diarrhea, dysentery, colic, and profuse sweating. It is in this stage of the infection, about 48 hours after ingestion of the larvae, that the disease must be differentiated from food poisoning. If a history of the eating of uncooked or improperly cooked pork is elicited, encysted nematodes may be sought in the food, if any is still available. There is no practical method for excluding trichina from pork at the time of slaughter, but thorough cooking removes the danger of infection. As the disease progresses, the diagnosis becomes more evident. When the larvae migrate into the tissues, patients may develop a severe myositis, spastic paralysis of the muscles, and edema of the face and hands.

Diagnosis depends on the stage of the disease. A marked eosinophilia is suggestive of trichinosis. During the intestinal phases, adult worms are sometimes recovered from the feces. During and after larval migration, the larvae are often found in muscle strips removed at biopsy. Various modifications of G. W. Bachman's (9) intradermal and precipitin tests with worm extracts are valuable, especially in the later stages of the disease.

EPIDEMICS OF ACUTE DIGESTIVE UPSETS OF VIRUS OR OF UNKNOWN ETIOLOGY

Epidemics of acute digestive upsets which cannot be associated with food or water have been reported in widely scattered localities (10, 11). In an outbreak of 41 cases (10) the onset was gradual in most individuals and began with nausea, vomiting, and diarrhea. The patients did not become ill at once, but over a period of hours. In some the illness was associated with colds, but in many there was no history of any upper respiratory infection. In 29 individuals from whom symptoms were tabulated, diarrhea, which occurred in 25 individuals, was the most common, nausea and vomiting were the next most frequent, and 13 had fever. Some of the patients remained ambulatory and were ill for only a few hours, whereas others were ill for from 2 to 3 days. There was some evidence that the illnesses were spread by contact or possibly by droplet infection, since they were limited to certain floors in 1 dormitory and 2 individuals who had had contact with those ill were similarly afflicted 4 days later. No intestinal pathogenic organisms were detected in fecal cultures. Furthermore, attempts to find a virus by inoculating monkeys (*M. mulatta*) intranasally with nasopharyngeal washings failed. Also 2 monkeys, injected intraperitoneally with citrated blood from 2 of the patients, did not develop a nasal discharge or diarrhea. Inasmuch as this outbreak occurred over a period of several days and was not associated with food or water, it could readily be differentiated from food poisoning.

J. A. Baker (12) isolated and described a filtrable virus which he believed was the cause of "scours" (pneumoenteritis) in cattle. Calves less than 1 month of age are susceptible, and the disease is characterized by fever, diarrhea, and pneumonia. F. L. Horsfall and R. G. Hahn (13) showed that it is often possible to demonstrate a latent infection in apparently normal mouse lungs by serial intranasal inoculations into normal mice at 7–9-day intervals. After the second or third serial passage of the inapparent infection, the latent virus acquires sufficient pathogenicity to produce typical pneumonia. Baker inoculated groups of virus-free young mice intranasally with 0.05 ml. of a 24 per cent suspension of pulmonary tissue from scours in calves. The initial group of mice did not show demonstrable signs or symptoms of disease. By the third serial passage from the apparently normal lungs, definite pulmonary lesions were produced, increasing to a lethal pneumonia by the eighth passage. Filtration experiments showed that the infectious agent in the mouse lungs readily passes through a Berkefeld N filter and is presumably a virus. Suspensions of these infected mouse lungs inoculated into calves produced fever (105.8° F.) and diarrhea after an incubation period of about 3 days, followed 2 days later with signs and symptoms of pneumonia. The diarrheal symptoms stopped about 3 days later, at which time the temperature became normal. When the infected calves were examined, the virus was found only in the lungs and intestine during the first day after onset of the fever. Three or 4 days later the virus was found in the serum, liver, spleen, kidneys, and mesenteric lymph nodes. The virus was neutralized by serum of calves recovered from experimental mouse virus infection or by serum of convalescent animals with the natural scours disease.

Two epidemics of widespread mild disease, characterized by anorexia, malaise, diarrhea, nausea, and vomiting, occurred in October and November of both 1943 and 1944 in Philadelphia and its environs and in other widely scattered places at the same times (14). Bacteriological study of the stools and pharyngeal secretions of 10 patients revealed no unusual bacteria or parasites to account for the disease. About 100 mice were injected; some were injected intranasally, rectally, or orally, with the supernatant fluid resulting from centrifuging diarrheal stool samples from 5 patients mixed with saline. Pharyngeal washings in bouillon were inoculated intranasally into mice and, after filtration, intracerebrally. Some mice developed a pneumonitis, and further lung passages were carried out in some cases to the fourth serial passage. From these studies no virus was isolated. Intranasal inoculations of stool filtrates into 8 calves, aged 4 weeks, were also negative.

Reimann and his associates (15) then resorted to experimentation on student volunteers. Suspensions of diarrheal stools and broth garglings were passed through a Mandler filter. The resulting filtrates were nebulized and passed into a large box into which the volunteer placed his head for 5 minutes. Of 21 students inhaling nebulized stool filtrates, 11 developed typical diarrheal symptoms after an incubation period of less than 4 days. In 3 cases the diarrhea was accompanied by a mild nasopharyngitis. Thirty-two students inhaled nebulized filtered garglings. Of these, 17 developed diarrhea without nasopharyngitis. No symptoms developed in 6 students who inhaled nebulized patient's serum. A group of 15 students swallowed 3 ml. of filtered garglings in double gelatin capsules. Five swallowed stool filtrates, and 4 patients' serums similarly encapsulated. No symptoms followed such oral administration. These investigations were carried out during the course of a natural epidemic without precautions for isolation, so that further work is necessary to evaluate the results properly.

In contrast to the work of Reimann and his associates is the work of Gordon *et al.* (16). These workers studied an epidemic disease which was present in several New York State institutions. It was characterized, clinically, by sudden onset, profuse diarrhea, usually by vomiting, often by cramps, headache, and dizziness. Fever was slight or absent, and no characteristic bacterial agents could be demonstrated. Recovery ensued in 48–72 hours, but at times required a week. Respiratory symptoms were not evident. Experiments were reported with 28 human volunteers. All subjects were kept under hospital isolation in isolation cubicles. Human volunteers developed gastroenteritis when fed fecal filtrates in capsules from cases of epidemic gastroenteritis. The incubation period ranged from 1 to 5 days, with a mean of 3 days. The disease was carried through three generations, in the last two by means of fecal filtrates. Oral administration of unfiltered throat washings from experimental cases of the disease induced gastroenteritis, but subjects inhaling a portion of the same throat washings remained asymptomatic. Embryonated hens' eggs were inoculated with one unfiltered stool suspension that had been used in a pool with another stool suspension which had induced gastroenteritis in each of 3 volunteers to whom it was fed. Three sets of eggs were inoculated: one on the chorio-allantoic membrane, another into the yolk sac, and a third into the amniotic sac. Streptomycin and penicillin were used as antibacterial agents. After three serial passages by each method, tissue and extra-embryonic fluids were noninfective for volunteers.

J. W. Smillie, B. F. Howitt, and G. A. Denison (17) studied an epidemic of acute watery diarrhea occurring in Jefferson County, Alabama. The incubation period for 40 cases based on known contact ranged from 1 to 12 days, with a median of 3 days. Environmental sanitation was good, and water, food, and milk did not appear to be sources of infection. The length of illness ranged from 1 to 14 days, with means of 5.1 days. There was no age group exempt, and both men and women were equally attacked. Numerous attempts were made to isolate a virus by various methods in both embryonated eggs and small animals, but no virus was found from either fecal specimens or nasal washings.

Although many outbreaks of epidemic diarrhea and vomiting are reported (18, 19, 20), there is reason to believe that the vast majority go unrecognized. Since this type of infection simulates the symptoms of food poisoning, it is important that it be differentiated from food poisoning. The most striking difference is due to the fact that epidemic diarrhea is not related to food or water and that an epidemic in a community may cover a period of many weeks. Certainly, it is of utmost importance to exclude these illnesses from the category of food-poisoning outbreaks.

CONCLUDING REMARKS

Symptomatology, epidemiology, pathology, and laboratory diagnosis are all essential in establishing a correct diagnosis in any outbreak of food poisoning. They must all be included in an intelligent analysis of such an outbreak. Where all are not considered, specific agents may be falsely incriminated. In this book a critical discussion is given of the various agents which may cause food poisoning, and it is hoped that the various similarities and distinctions which have been drawn will be helpful to individuals engaged in studying the numerous gastrointestinal upsets which may or may not be due to food poisoning.

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AUTHOR INDEX

[Numbers in parentheses are reference numbers listed at the ends of the respective chapters.]

Abel, J. J., 37, 41 (25)
Abrahamson, A. E., 157 (6), 161
Abrams, A., 74 (84), 75 (93), 78, 79
Aleppo, P. L., 140 (55), 145 (54) (55)
Allen, Rex W., 20 (16), 24, 50 (12), 51 (12), 76
Aoki, K., 146 (3), 154
Atkinson, J. D., 90 (30), 100 (30), 117
Austen, F. E., 132 (25), 144

Bachman, G. W., 166, 171 (9)
Baerthlein, Karl, 86 (13), 116, 157 (7), 161
Bahr, L., 141 (67), 142, 145
Baker, Henry B., 84
Baker, J. A., 167, 171 (12)
Baldwin, H. B., 17, 24 (11)
Bancroft, H., 164 (5), 170
Barber, M. A., 85, 86, 87, 105, 110, 116 (12)
Barger, 38, 44 (28)
Barnes, L. A., 157 (8), 160 (15), 161
Bartram, M. T., 130, 131 (22) (23), 144 (22)
Bass, K., 163 (4), 170
Bayliss, M., 115, 116, 118 (80), 134 (33), 144
Beamer, P. R., 69 (66), 78
Beck, D., 68 (61), 78
Beeman, J. A., 18 (13), 24
Bellamy, W. D., 153 (13), 154
Bengston, I. A., 60 (34), 61 (38), 67 (55), 71, 76, 77, 78
Benson, H. W., 40 (35), 41
Bergmann, Max, 115 (79), 118
Beveridge, G. W., 75 (91), 79
Bishop, G. H., 53 (19), 76
Boehm, 39
Borman, E. K., 134 (32), 141 (62), 144, 145
Bornstein, S., 134 (31), 144
Bowman, G. W., 107 (70), 118
Branham, S. E., 141 (66) (70), 145
Breiffelh, L. A., 123 (11), 143
Broh-Kahn, R. H., 140 (56), 145
Bronfenbrenner, J. J., 53 (19), 76
Brown, E. G., 123 (9), 130 (9), 143
Brown, G., 170 (18), 171
Brown, J. H., 146 (1), 153
Bruner, D. W., 134, 136 (43), 137, 138 (30), 139 (30), 140 (59), 141 (59), 144 (30) (45) (49), 145
Buchbinder, L., 149, 150, 154
Buckenham, John, 81, 116
Buehler, H. J., 74 (89), 79
Burke, G. S., 68 (61), 78
Burrows, W., 109, 113, 115, 118

Caldwell, M. E., 141, 145 (60)
Campbell, P. S., 28
Caprio, F. S., 52 (14), 76
Carr, J. L., 19 (14), 24
Cary, W. E., 51 (13), 76, 86, 96, 97 (42), 111 (42), 117 (42), 141 (68), 142 (74), 145, 146 (7) (8), 154, 160 (13), 161
Castellani, 137
Caudill, F. W., 88 (17), 117
Chalmers, 137
Chapman, G. H., 103, 118 (64)
Chemerda, C., 135, 144 (36)
Cherry, W. B., 128, 144 (21), 157 (8), 160 (15), 161
Chinn, B. D., 103, 118 (63)
Clegg, H. W., 88 (21), 117
Cockcroft, W. H., 96, 111, 117 (44)
Cogswell, W. F., 91 (37), 117
Coleman, G. E., 62 (42*f*), 73, 77, 78 (80)
Coleman, M. B., 99, 118 (52), 159 (12), 161
Collier, F. C., 28 (3), 40
Combs, G. R., 123 (9), 130 (9), 143
Cooper, K. E., 156 (5), 161
Coriell, L. L., 75 (91), 79
Couch, J. F., 35 (19), 41, 72, 78 (77)
Coughlin, F. E., 89 (27), 117
Crawford, G. J., 170 (18), 171
Culley, A. R., 123 (8), 126, 130 (8), 143

Cummings, J. R., 67 (58), 71 (58), 78
Curcio, L. G., 103, 118 (64)

Dack, G. M., 54 (22), 57 (26) (27) (28), 60 (32) (36) (37), 64 (43) (44), 65 (49), 66 (49) (51), 67 (51), 73, 76, 77, 78, 86, 88, 91, 94 (39), 95 (39), 96, 97 (42), 99, 101 (54) (55), 102 (49), 104 (15), 106 (49), 107 (70), 108 (15) (71), 109 (49), 110 (33) (74), 111 (42), 112 (75) (76), 113 (76), 114, 117 (15) (42), 118 (49) (50), 125, 132 (28), 133 (29), 140 (57), 141 (63) (64) (68), 142 (73) (74), 144 (17), 145, 146 (7) (8), 152 (11), 154, 158 (9) (10), 160 (13), 161, 163 (3), 166 (11), 170, 171
Davies, J., 156 (5), 161
Davison, E., 72, 96, 97 (42), 101 (54), 108 (72), 111 (42), 112 (76), 113, 114, 115, 117 (42), 118, 141 (63), 145, 146 (8), 154
Day, L. A., 141 (70), 145
Deadman, W. J., 91 (36), 117
DeLay, P. D., 88 (25), 117
Denison, G. A., 90, 117, 169, 171 (17)
Denys, 85, 90, 116 (10)
Dickson, E. C., 43 (3), 44 (5), 45, 51 (5), 52, 53 (5) (16) (17), 57 (5), 58 (3) (5), 68 (62), 76, 78
Dolman, C. E., 91 (35), 96, 103, 111, 117 (44), 118 (61)
Donaldson, R., 2 (2), 11
Dornicka, C. G., 23 (26), 25
Doull, J. A., 164 (5), 170
Doyle, H. S., 88 (24), 117
Doyle, L. P., 139 (53), 144
Dragstedt, L. R., 57 (27), 77, 158 (9) (10), 161
Dubovsky, B. J., 62, 63, 64, 66 (42*e*), 77
Dyssegaard, A., 141 (67), 142, 145

Ecklund, A., 50 (12), 51 (12), 76
Edmunds, C. W., 53 (18), 76
Edwards, P. R., 123 (13), 134, 136 (43), 137, 138 (30), 139 (30), 140 (59), 141, 143, 144 (30) (45) (49), 145
Eklund, H. W., 74 (85) (86), 78
Elford, W. J., 115, 118
Elkeles, G., 141, 145 (72)
Elliot, F. J., 91 (36), 117
Elvehjem, C. A., 21 (21), 25
Enzie, F. D., 20 (16), 24
Erickson, T. C., 21 (21), 25
Ermengem, E. van, 43, 120, 122, 143 (3)
Esty, J. R., 59 (31), 66 (53) (54), 67 (53), 68 (53), 77

Faust, H., 69 (64), 78
Favorite, G. O., 94, 97, 117 (40)
Fisk, R., 60, 77
Ford, W. W., 37, 38, 41 (22) (24) (25) (27)
Foster, A. O., 20 (16), 24
Foster, Jackson W., 69 (67), 70 (68), 78
Frant, S., 16, 24 (8), 157 (6), 161
Fraser, H. F., 163 (2), 170
Friewer, F., 163 (4), 170
Fruton, J. S., 115 (79), 118
Fuchs, A. W., 88, 89 (26), 117 (26)

Garber, J. S., 16 (9), 24
Garlock, F. C., 140 (56), 145
Gärtner, A., 120, 129, 138, 143 (2)
Geiger, J. C., 12, 18, 24 (2) (12), 42 (1), 44 (5), 45, 51 (5), 52 (1), 53 (5), 57, 58 (5), 67 (1), 76, 141 (69), 145
Gershoff, S. N., 21 (21), 25
Gibbard, J., 28 (3) (4), 40, 57 (28), 77
Gibbons, N. E., 135, 144 (35)
Gilbert, R., 159 (12), 161
Gilcreas, F. W., 99, 118 (52)
Gilman, Robert L., 32, 41 (14)
Gilson, W. E., 21 (21), 25
Giltner, L. T., 72, 78 (77)
Glassman, H. N., 74 (90), 79
Gompertz, L. M., 38, 39, 41 (29)
Gordon, I., 169, 171
Gorrell, F. E., 22 (23), 25
Gowen, G. H., 36, 41 (21)
Graham, R., 58 (29), 77
Gray, J. P., 166 (10), 171
Greenwood, D. A., 17 (10), 24
Gunnison, J. B., 61 (39), 67 (56) (58), 71 (56) (58), 72 (79), 77, 78
Gunsalus, I. C., 153 (13), 154
Guyton, A. C., 53 (20), 76

Habermann, R. T., 20 (16), 24
Haerem, S., 57 (27), 77
Hahn, R. G., 167, 171 (13)
Hall, I. C., 48, 76 (9) (10)
Halliday, E. I., 108 (71), 118
Halvorson, H. O., 102 (60), 118
Hammerschmidt, J., 126 (18), 143, 144

Hammon, W., 94, 97, 98, 111, 113, 114, 115, 117 (40), 118 (48)
Harger, R. N., 88 (20), 107 (70), 117, 118
Hargreaves, E. R., 170 (19), 171
Harmon, P. H., 125, 136 (39), 141 (68), 142 (74), 144 (17), 145, 160 (13), 161
Harris, N. M., 36, 41 (20)
Hata, M., 88 (18), 89 (18), 93 (18), 117
Hathaway, J. S., 170 (20), 171
Haynes, W. C., 104 (66), 118
Hazen, E. L., 67 (57), 71 (74), 78
Heinz, T. E., 158 (10), 161
Hemmens, E., 65 (49), 66 (49), 77
Henderson, L. L., 134 (34), 144
Henderson, N. D., 132 (24), 144
Hill, E. V., 74 (83), 78
Hill, I. B., 18 (13), 24
Hinshaw, W. R., 135, 144 (37)
Hintze, K., 1 (1), 11
Hite, K. E., 97, 109, 117 (45)
Hodges, J. H., 168 (14) (15), 171
Hoeft, G. L., 165 (7), 171
Hogan, W. A., 97 (47), 118
Hopkins, E. W., 98
Hormaeche, E., 127, 128 (19), 138, 140 (55), 144 (19) (48), 145 (54) (55)
Horsfall, F. L., 167, 171 (13)
Hoskins, D., 57 (26), 76
Hottle, G. A., 74 (84), 75 (91) (92) (93), 78, 79
Houser, G. H., 123 (11), 143
Housewright, R. D., 75 (94), 79
Howitt, B. F., 169, 171 (17)
Hucker, G. J., 104, 118
Humphrey, E. C., 88 (17), 117
Hunter, A. C., 48 (8), 71 (8), 76
Hunter, C. A., 49 (11), 51, 76
Hunter, F. R., 110 (74), 118
Hutton, J. E., 33 (17), 35, 41

Ingraham, H. S., 169 (16), 171
Irons, E. E., 155, 161 (1) (2)
Isaacs, M. L., 101 (57), 118

Janota, M., 163 (3), 170
Jarra, J. E., 125, 144 (17)
Jensen, L. B., 65 (47), 66 (50), 77
Johnson, B., 89 (27), 117
Johnson, J., 68 (61), 78
Johnson, R. E., 21 (20), 24
Jones, A. H., 97 (47), 101, 118
Jones, L. D., 20 (16), 24
Jordan, E. O., 36, 41 (20), 62, 64, (43), 77, 91 (33), 95, 103, 104, 109, 110 (33), 112 (75), 113, 115, 117 (41), 118 (65), 136 (39), 139, 142 (73), 144, 145, 155, 161 (1) (2)
Judefind, T. F., 140 (58), 145
Jungewaelter, F. G., 99, 118 (50)

Kadavy, J. L., 65 (49), 66 (49), 77
Kadull, P. J., 75 (94), 79
Kahns, E., 91 (37), 117
Kalmbach, E. R., 71, 72, 78
Kark, R. M., 21 (20), 24
Kauffmann, F., 136, 137 (40), 138 (40) (47), 144
Kegel, A. H., 20 (17), 24
Kegeles, G., 74 (84) (87), 78
Kelly, F. C., 88, 91 (15), 104 (15), 108 (15), 117 (15)
Kershaw, G. R., 3 (3), 11
Kilbourne, B. R., 91 (37), 117
King, H., 68 (61), 78
Kiralis, E. S., 30 (8), 41
Kleeman, I., 16, 24 (8), 157 (6), 161
Klein, J. R., 21 (20), 24
Knight, Henry G., 22 (23), 25
Kob, M., 60, 77 (33)
Kobert, 37, 41 (23)
Kodama, T., 88 (18), 89 (18), 93 (18), 117
Kofoid, C. A., 29 (5) (6), 30 (5), 40
Kolesnikova, M. K., 54, 76 (24)
Koong, C. H., 165 (7), 171
Korff, F. A., 99, 118 (51)
Korns, R. F., 169 (16), 171
Koser, S. A., 44 (7), 50 (7), 58 (7), 60 (7), 76, 161
Kupchik, G. J., 97, 103, 117 (46)

Lamanna, C., 73 (82), 74 (85) (86) (88) (89) (90), 78, 79
Laviano, A. B., 159 (12), 161
Leffmann, Henry, 83, 116
Leighton, G., 58 (30), 77
Leim, A. H., 28 (4), 40
Lentz, P. L., 157 (8), 161
Levinson, S. O., 163 (4), 170
Lewis, C., 73 (82), 78
Lewis, K. H., 74 (83), 78
Lichty, J. A., 75 (92), 79

Lidbeck, W. L., 18 (13), 24
Lieb, C. W., 103, 118 (64)
Lindberg, R. B., 134 (33), 144
Linden, B. A., 88 (16), 89, 117, 146, 154 (5)
Litterer, W., 123 (6), 130 (6), 139 (6), 143
Lockheed, A. G., 97 (47), 101, 118
Lodenkämper, H., 156, 161 (3)
Long, P. H., 53 (18), 76
Luff, M., 23 (24), 25
Luisada, 33 (16), 41
Lumsden, L. L., 88 (23), 117

McBroom, J., 95, 117 (41)
McBurney, R., 91 (34), 117
McCallum, G. L., 132 (24), 144
McCastline, W. B., 101 (57), 118
McCaughey, M. B., 30 (9), 41
McClung, L. S., 160 (17), 161
McClure, W. B., 90 (31), 100 (31), 117
McCrae, T., 34, 35, 41 (18)
MacDonald, M. A., 53 (20), 76
McElroy, O. E., 74 (85) (86), 78
McFarlane, V. H., 135, 144 (36)
McIlhenny, M., 165 (7), 171
Mackenzie, G. M., 67 (59), 78
Mackey, Edward, 82, 116
McNally, W. D., 12 (1), 16 (1), 20 (17), 24
McNeil, E., 135, 144 (37)
Mallman, W. L., 139 (52), 144
Mann, W. L., 32 (15), 41
Matthews, Evelyn, 139 (52), 144
Medcof, J. C., 28 (4), 40
Mellanby, E., 21 (19), 24
Meyer, K. F., 26 (2), 28, 29 (7), 31, 40, 43 (2), 44 (5), 45, 51 (5), 53 (5), 57 (5), 58 (5), 59 (31), 60 (4), 61 (39) (41), 62, 63, 64, 66 (42*e*) (53), 67 (53) (56) (58), 68 (53) (63), 71 (56) (58), 72 (79), 73, 75, 76, 77, 78 (80), 141 (69), 145
Michael, A. C., 88 (20), 117
Mickle, F. L., 134 (32), 141 (62), 144, 145
Miller, A. M., 90 (31), 100 (31), 117
Minett, F. C., 96, 113, 115, 177 (43)
Mitchell, R. B., 140 (56), 145
Mold, J. D., 30 (8) (9) (10) (11), 41
Monahan, E. P., 21 (20), 24
Monnet, R., 16 (7), 24
Monnier, R. P., 30 (8), 41
Moore, R. L., 135, 144 (35)
Moran, A. B., 137, 144 (44)
Morton, W., 15 (5), 24
Myers, E., 146 (7), 154

Nau, C. A., 88 (23), 117
Naubert, J., 28 (4), 40
Needler, A. B., 28 (4), 40
Needler, A. W. H., 28 (4), 40
Nelson, C. I., 160 (16), 161
Nelson, N. A., 146 (4), 154
Newell, G. W., 21 (21), 25
Nigg, C., 75 (91) (92), 79
Niven, C. F., Jr., 146, 154 (6)
Noble, I., 108 (71), 118
Nocard, 137
Novy, 85

Oddy, J. G., 88 (21), 117
Oettingen, W. F. von, 20, 24 (18)
Oglesby, E. W., 69 (65), 78
Ollivier, M., 81
Olson, C. L., 49 (11), 51 (11), 76
Olsson, R. C., 163 (4), 170
Osler, A. G., 149 (9), 150 (9), 154
Ostrolenk, M., 130, 131 (22) (23), 132 (27), 144 (22)
Owen R. W. G., 85, 116 (11)

Parker, R. F., 164 (5), 170
Pasteur, L., 80, 116
Pawlowsky, E. N., 39 (30), 41
Peluffo, C. A., 138, 140 (55), 144 (48), 145 (54) (55)
Perch, Beate, 123 (12), 143
Petran, E. J., 170
Pfenninger, W., 61 (40), 73, 77
Phillips, A. W., Jr., 101 (56), 102 (56), 118
Pilcher, R. W., 65 (48), 77
Poland, E. F., 98
Pond, M. A., 170 (20), 171
Pope, A. S., 20 (17), 24
Price, A. H., 168 (14) (15), 171
Proctor, B. E., 101 (56), 102 (56), 118
Putnam, F. W., 74 (88), 79

Read, M. R., 164 (5), 170
Reames, H. R., 75 (94), 79

Reed, J. M., 68 (60), 78
Reimann, H. A., 168 (14), 171
Reiter, O., 44 (7), 50 (7), 58 (7), 60 (7), 76
Rickher, C. J., 69 (66), 78
Riegel, B., 30 (8) (9) (10) (11), 41
Rigdon, R. H., 97 (47), 118
Robb, H. F., 40, 41 (34)
Roberts, J., 91 (36) (38), 100, 117, 118 (53)
Robey, L., 141 (70), 145
Robinson, 70, 78 (72) (73)
Roholm, K., 19 (15), 24
Rosenau, M. J., 24 (27), 25, 123 (4), 126, 130 (4), 143
Rosenbach, F. J., 80 (2), 116
Rosenwald, A. S., 75 (91), 79
Rubin, H. L., 127, 128 (20), 144 (20)
Rustigian, R., 160 (14), 161
Ryerson, D. L., 141, 145 (60)
Ryff, J. F., 139 (52), 144

Sabon, F., 16 (7), 24
Sakai, 146 (3), 154
Salmon, D. E., 119, 138, 143 (1)
Salsamendi, R., 127, 128 (19), 144 (19)
Saphra, I., 134 (31), 141, 144, 145 (61)
Savage, W. G., 125, 126, 141 (71), 144 (16), 145 (65), 156, 158, 161 (4)
Schantz, E. J., 74 (89), 79
Scherage, M., 127, 128 (20), 144 (20) (21)
Schiff, F., 139 (51), 144
Schlesinger, H., 38, 41 (27)
Schneider, H. J., 60 (35), 77
Schönberg, F., 158 (11), 161
Schoenholz, P., 26 (1), 28 (1), 40, 59 (31), 62 (42*g*), 63 (42*g*), 76, 77
Schutze, H., 138 (46), 144
Schwarze, H., 58 (29), 77
Schytte, B. A., 123 (7), 130 (7), 138 (7), 143
Scott, W. J., 70 (69) (70), 78
Seddon, 73, 76
Segalove, M., 99, 101 (54), 102 (49), 106, 107, 109 (49), 118 (49) (69), 133 (29), 144, 152 (11), 154
Seibert, 38, 41 (26)
Seligmann, E., 141, 145 (61)
Selmi, 1
Sharp, D. G., 74 (88), 79
Shaughnessy, H. J., 163, 170
Shaw, R. M., 72 (78), 78
Sherman, J. M., 146, 152 (12), 153, 154 (6)
Shevky, R., 53 (16) (17), 76
Sibuya, Y., 88 (18), 89 (18), 93 (18), 117
Silver, M. L., 21 (20), 24
Simpson, G. S., 72 (78), 78
Singer, A., 97 (47), 118
Slocum, G. G., 48 (8), 71 (8), 76, 88 (16), 89, 117
Smiley, D. F., 90, 106, 117 (29)
Smiley, K. L., 146, 154 (6)
Smillie, J. W., 169, 171 (17)
Smith, Theobald, 119, 138, 143 (1)
Smith, W. W., 20, 24 (18)
Soloviev, M. N., 44 (6), 76
Solowey, M., 135, 144 (36)
Sommer, H., 26 (1) (2), 28 (1), 29 (5) (7), 30 (5) (8) (9), 31 (13), 40, 41
Spaulding, E. H., 135, 144 (36)
Spray, R. S., 123 (5), 130 (5), 143
Stanger, D. W., 30 (8) (9) (10) (11), 31 (12), 41
Starin, W. A., 60 (36) (37), 66 (52), 73, 77, 78 (81), 107
Stead, F. M., 88 (23), 117
Steffen, G. I., 149 (9), 150 (9), 154
Stent, L., 170 (18), 171
Sternberg, George M., 84, 116
Steward, H. C., 123 (6), 130 (6), 139 (6), 143
Stewart, D. F., 70 (69) (70), 78
Stohler, R., 29 (5), 30 (5), 40
Stone, R. V., 103, 104, 109 (62), 118 (62)
Strauss, L., 134 (31), 144
Stritar, J., 99, 104, 118 (50) (65)
Stuart, C. A., 160 (14), 161
Surgalla, M. J., 97, 101 (55), 104, 109, 117 (45), 118 (67), 132 (28), 144, 152 (11), 154
Sutton, C., 124, 126, 142, 143 (14)
Swanson, H., 52 (15), 76
Swift, S., 88 (22), 90 (22), 100 (22), 101 (22), 117

Tanner, F. W., 66 (51), 67 (51), 69 (65) (66), 77, 78, 101 (59), 118
Taylor, T. J., 135, 144 (37)
Tesdal, M., 123 (7), 130 (7), 138 (7), 143
Theiler, 70, 78 (71) (72) (73)

Thom, C., 146, 154 (5)
Thompson, R., 101 (57), 118
Timmerman, W. A., 88 (19), 89, 117
Tipton, V. C., 90, 106, 117 (29)
Tonney, F. O., 165 (7), 171
Topley, W. W. C., 161
Topping, N. H., 163 (2), 170
Torda, C., 53 (21), 76
Trenting, W. L., 123 (11), 143
Tucker, C. B., 52 (15), 76
Turner, W. R., 146, 154 (5)

Ullery, J. C., 34, 35, 41 (18)
Ulrich, J., 102 (60), 118

Vaughan, Victor C., 83, 85, 116
Velikanov, I. M., 54, 76 (24)
Verder, A. E., 124, 126, 142, 143 (14), 163 (1), 170
Vollum, R. L., 88 (22), 90 (22), 100 (22), 101 (22), 117

Walkey, F. L., 139 (53), 144
Wassermann, M., 141, 145 (61)
Watson, W. E., 56, 76 (25)
Watt, James, 135, 144 (38)
Weaver, R. H., 127, 128 (20), 144 (20) (21)
Weed, L. A., 88 (20), 117
Weiss, H., 123 (4), 126, 130 (4), 143
Weiss, J. E., 49 (11), 51 (11), 76
Welch, H., 48 (8), 71 (8), 76, 130, 131 (22) (23), 132 (27), 144 (22)
West, D. E., 134 (32), 141 (62), 144, 145
West, M. G., 140 (59), 141 (59), 145
Wheaton, Evan, 65 (48), 77
Whedon, W. F., 29 (5) (6), 30 (5), 40
Wheeler, K. M., 134 (32), 141 (62), 144, 145
White, P. B., 125, 126, 136, 141, 144 (16) (41), 145 (65)
Whyte, E. F., 28 (3), 40
Wiggers, H., 86 (14), 117
Wikholm, D. M., 30 (8) (9) (10) (11), 41
Williams, G. C., 88 (22), 90 (22), 100 (22), 101 (22), 117
Williams, H., 12 (3), 24
Williams, O. B., 68 (60), 78
Wilson, G. S., 88 (22), 89, 90 (22) (30), 100 (22) (30), 101 (22), 117 (28), 161
Wilson, J. B., 75 (94), 79
Wilson, R. L., 91 (38), 96, 99, 100, 111, 117 (44), 118
Wiseman, J., 156 (5), 161
Wolff, A. H., 132, 144
Wolff, H. G., 53 (21), 76
Wood, W. L., 54 (22), 76, 142 (73), 145
Woolpert, O. C., 54 (23), 76, 86 (14), 91 (33), 94 (39), 95 (39), 108 (71), 110 (33), 112 (75), 117, 118
Wright, E., 123 (9), 130 (9), 143
Wynne, E. Staten, 69 (67), 70 (68), 78

Ziven, S. S., 21 (20), 24
Zlatogoroff, S. I., 44 (6), 76

SUBJECT INDEX

Agene, 21–22
Alkali poisoning, 36
Allantiasis, 43
Allergy, 35
Alouta palliata inconsonans, 95
Aluminum, 13
Amanita citrina, 38
"Amanita hemolysin," 38
Amanita muscaria, 38
Amanita phalloides ("deadly Amanita," "white Amanita"), 27, 37, 38
Amebic dysentery, 11, 156, 165
Anthelmintic, 20
Antimony poisoning, 8, 13, 14
  incubation period of, 13, 14
  source of, 8, 13, 14
  symptoms of, 13, 14
Antipasto (Italian), 47
Aplopappus heterophyllus, 36
Apples
  cooked, 14, 23
  raw, 13
  sauce, 23, 46
Apricot butter, 46
Apricots, home-canned, 45, 46, 48
Arsenic poisoning, 8, 12, 13, 15
  incubation period of, 14, 15
  source of, 8, 13, 15
  symptoms of, 14, 15
Ascaris lumbricoides, 20
Asparagus
  commercially canned, 22, 59, 70, 101, 132, 152
  home-canned, 45, 46, 51
Ateles ater, 5
Ateles geoffroyi, 95

Bacillary dysentery, 11, 162–64
Bacillus botulinus, 63
Bacillus enteritidis, 120
Bacon, 65, 81
Bacterial food poisoning
  general, 9–11, 153
  miscellaneous, 11, 155–61
Bacteriophages, 90, 100
Bacterium aerogenes, 109
Bacterium coli, 11, 89, 109, 124, 142, 166
Bakery goods
  cream-filled, 10, 83, 88, 89, 100, 159
  custard-filled, 88, 89, 99, 100, 108, 109
Baking powder, 8, 12, 14
Baking soda, 8, 14, 17, 18
Barium carbonate poisoning, 14, 15
  incubation period of, 14, 15
  source of, 14, 15
  symptoms of, 14
Beans, 46, 70
  commercially packed, string, 46, 47, 59, 101, 132, 152
  home-canned, string, 45, 46, 47, 49, 50, 51
Beef, 47, 65, 85, 88, 90, 101, 132, 147, 148, 150, 151
  tamales, 47
Beets, 46, 59, 70
Benzoyl peroxide, 21
Bologna, 47
  ham, 150
Borax, 24
Boric acid, 24
Botulism, 2, 10, 20, 42–79, 119, 153
  Clostridium botulinum, the agent in, 60–76, 153
    effect of moisture and sodium chloride on, 65
    factors effecting growth of, 69–70
    pathogenicity of, 70–73
    resistance of spores of, to heat, 66–69
    in the soil, 61–64
    toxin of, 42, 43, 73–76
  control of, 60–76
  foods involved in, 10, 44–49
  of fowl (limber-neck), 58
  geographic distribution of, 44–45
  history of, 43–44
  incubation period of, 10, 153
  laboratory diagnosis of, 58–60
  mortality and pathology of, 57–58
  outbreaks of, 44–45
  symptoms of, 10, 49–53, 153
  treatment of, 52, 54–57
Botulismus, 43
Botulus, 43
Brawn, 156
Bread, 108
Buffalo meat, 47

Cabbage, 70
Cadmium poisoning, 8, 14, 16–17
  food involved in, 8, 14, 16
  incubation period of, 8, 14
  source of, 8, 14, 16
  symptoms of, 8, 14, 16
Cake, sponge, 86, 87
Calf's head vinaigrette, 47
California Olive Association, 44
California State Department of Health, 26
Canners League of California, 44
Canning, 10, 42, 49, 50, 68
Caranx, 33
Carrots, 46, 70
Cauliflower, 46, 70
Cebus capucinus capucinus, 95
Celery, 46
Chard, 46
  swiss, 70
Charlotte russe, 150
Cheese, 47, 81, 83, 84, 88, 89, 123, 130
  poisoning by, 85
Chemical poisoning
  general, 8, 12–25
  incubation period of, 8
  sources of, 8
  symptoms of, 8
Chicken, 47, 88, 101, 104, 160
Chili, 46, 48
Chili con carne, 47
Chlorine, 21
Chlorine dioxide, 21
Cholera, 162
Cicuta maculata (wild carrot, wild parsnip, parsnip), 27, 38, 39
Cicutoxin, 39
Clams, 31, 47
Claviceps purpurea, 9, 27, 38
Cloacae-aerogenes group, 159
Clostridium botulinum; see Botulism
Clostridium parabotulinum, 61, 70
Clostridium sporogenes, 2, 62, 64, 65
Clostridium welchii, 160–61
Colon aerogenes group, 141
Cooking utensils
  cadmium plated, 8, 14, 16, 17
  galvanized iron, 14, 23
  gray-enameled, 8, 14
Copper, 13
Corn
  commercially packed, 46, 59, 101, 108, 133, 152
  home-canned, 45, 46, 50
Crab, 47
Cream, 105
Cucumber, 46
Curare, 53
Cyanide poisoning, 12

Decalso, 30
Diatoms, 29
Dinoflagellates, 29
Drinker respirator, 52
Duck paste, 58
Duck sickness, 71, 72
Dysentery, 141; *see also* Bacillary dysentery; Amebic dysentery

Éclairs, 99
  chocolate, 88, 159
  cream-filled, 88
Egg salad, 136
Eggplant, 46
Eggs, 18, 123, 140
  dried, 134, 135, 149
  rotten, 2
Endamoeba histolytica, 165
Ergotism, 9, 38
  food concerned in, 9, 27, 38
  incubation period of, 27
  specific agent of, 9, 27, 38
  symptoms of, 27
Escherichia, 160
Eupatorium urticaefolium, 27, 35

Fava beans, 9, 26, 33, 35
Favism, 9, 33–35
  food concerned in, 9, 27, 33
  incubation period of, 27
  individual susceptibility to, 35
  specific agents of, 9, 27, 33
  symptoms of, 9, 27, 33, 34
Figs, 46
Fish, 44
  frozen, 101
  pickled, 47
  rotten, 1
  smoked, 157
Fish poisoning, 32–33
  etiology of, 32, 33
  incubation period of, 32
  symptoms of, 32
Flour, 8, 14, 15
  agenized, 21–22
Fluoride poisoning, 8, 12, 17–20
  dosage in, 17, 18, 19, 20

food concerned in, 8, 17
incubation period of, 8, 12, 14, 18, 20
pathology of, 19, 20
sources of, 8, 12, 14, 17, 18
symptoms of, 8, 14, 18, 19

Food and Drug Act of 1906, 24

Foods
canned, 10, 23
frozen, 101, 102
underprocessed, 42

Formaldehyde, 24

Frankfurter sausage, 47

Fruit, sprayed, 13

Fruit growers, 13

Gelatin, dessert, raspberry, 16

Gonyaulax catenella, 29, 30

Ham, 43, 65, 81, 88, 89, 101, 106, 108, 157
products, 47, 101, 104

Hepatitis, infectious, 164–65

Herring, 47

Hollandaise sauce, 88

Home brew, 47

Honey poisoning (poison honey), 9, 39

Hooper Foundation, University of California, 29, 62

Hormel Institute, 102

Hydrogen peroxide, 24

Ice cream, 85, 88, 101, 156

Ichthyosismus, 43

Insecticide, 8, 15, 17

Investigation, 2–11

Jimmy weed, 36

Kamaboko (fish sausage), 88, 89, 93

Lamb stew, 47

Lamziekte, 70

Lead poisoning, 8, 13, 15
incubation period of, 14, 15
source of, 8, 13, 15
symptoms of, 14, 15

Lemonade, 16

Liver, beef, 107, 108

Liver sausage, 47, 65, 88, 89

Macaca mulatta, 95, 139, 147, 158, 163, 167

Mangoes, 46

Mastitis, 86, 91

Mayonnaise, 104

Meat
canned, 47
cured, 10, 104, 109
dehydrated, 65
potted, 101, 132
preserved, 44
spoiled, 1, 2, 10

Meat inspection, 129

Meat products, 128

Mercuric chloride, 21

Mercury poisoning, 8, 14, 21
incubation period of, 14
sources of, 8, 21
symptoms of, 14

Methyl chloride poisoning, 14, 20–21
dosage in, 20, 21
incubation period of, 14
source of, 14, 20
symptoms of, 14, 20

Methyl dichloramine, 21

Milk, 10, 27, 85, 86, 88, 90, 105, 110, 123, 130, 156, 160
canned, 45, 150
goat's, 88
powdered, 18

Milk sickness; *see* Snakeroot poisoning

Modiola modiolus, 28

Musca domestica, 132

Muscarine, 38

Mushroom poisoning, 9, 27, 37–38
food concerned in, 27, 37
incubation period of, 9, 27, 37
specific agent of, 27, 37, 38
symptoms of, 9, 27, 37
treatment of, 38

Mushrooms and mushroom sauce, 47, 67

Mussel poisoning; *see* Shellfish poisoning

Mussels, 8, 26, 27

Mutton, 123

Mytilus californianus, 26

Mytilus edulis, 28

National Canners Association, 42, 44

National Research Council, Food and Nutrition Board, 22

Nickel, 13

Nitrate poisoning, 14, 22
dosage in, 22
incubation period of, 14
sources of, 14, 22
symptoms of, 14, 22

Nitrogen dioxide, 21

Nitrogen trichloride (Agene), 21–22

Noodles, 101

Okra, 46
Olives, commercially canned, 42, 44, 45, 46
Onions (shallots), canned, 44, 46, 50, 58
Oxalate poisoning; *see* Rhubarb poisoning
Oxalic acid, 40
Oysters, 108

Pancakes, 17
Paracolon organisms, 141, 160
  Arizona group, 140
Paratyphoid organisms, 87, 119
Par-par, 46
Parsnips, 70
Pasteurization, 36
Pastry, 89
Peaches, canned, 46, 133, 152
Pears
  home-canned, 45, 46, 48
  sprayed, 15
Peas, 46, 59, 70, 101, 133, 152
Peppers, 46
Persimmons, 46
Phallin, 37
Phalloidine, 27
Pickles, 46
Pigsfeet, pickled, 47
Pies, 89
  cream, 100, 106, 147
Pimiento loaf, 46, 47
Pineapple trifle, 88
Plankton, 27, 29
Popsicles, 16
Pork, 11, 65, 89, 166
Pork products, 47, 82, 89, 123
Potatoes, sweet, 59, 70
Potassium permanganate, 24
Preservation of foods, 24
Proteus, 11, 109, 142, 156–59
Proteus mirabilis, 157
Proteus vulgaris, 86, 156–57
Psittacosis, 137
Ptomaine poisoning, 1, 2, 80
Pudding
  bread, 88, 123
  corn, 160
  rice, 123
Pumpkin, canned, 22, 46, 59
Punch, homemade, 16
Putrefaction, 1–2, 10

Questionnaire, 4–7

Rabbit pie, 81
Rat poison, 15
Rat "virus," 123, 131
Refrigerators, mechanical
  ice trays, 16
  leaking, 14, 20
Rhubarb, canned, 40
Rhubarb leaves, 9, 26, 39
Rhubarb poisoning (oxalate poisoning), 9, 39–40
  food concerned in, 9, 39, 40
  incubation period of, 40
  specific agent of, 40
  symptoms of, 40
  treatment of, 40
Roach powder, 18
Rye meal, 27

Salad dressing (mustard), 47
Salicylic acid, 24
Salmon
  canned, 47, 59, 101, 108, 109, 133, 152
  creamed, 101
  eggs, 47
  smoked, 67
Salmonella aberdeen, 139
Salmonella aertrycke, 109, 136, 137
Salmonella anatum, 127, 134, 135, 137, 139
Salmonella arizona, 141
Salmonella bareilly, 127, 134, 135
Salmonella berta, 123
Salmonella bovis-morbificans, 133
Salmonella bredeney, 127, 132
Salmonella california, 139
Salmonella cambridge, 137
Salmonella Center
  International, 136
  National, 136
Salmonella cerro, 132
Salmonella cholerae-suis, 123, 127, 133, 136, 138, 139
Salmonella cubana, 132
Salmonella derby, 127
Salmonella dublin, 133
Salmonella enteritidis, 121, 123, 127, 130, 132, 133, 134, 136, 138, 142, 143
Salmonella give, 127, 132, 134, 139
Salmonella hvittingfoss, 139
Salmonella illinois, 132
Salmonella infection, 10, 11, 87, 88, 89, 93, 119–45, 147, 149, 152, 153, 157, 158, 164

  agents in, 10, 133–41, 153
  characteristics of outbreaks of, 119–22
  control of, 127–33
  incubation period of, 11, 122–23, 153
  laboratory diagnosis of, 124–27
    selective media for, 124–25
  pathology of, 121–22
  symptoms of, 10, 119, 123–24, 153
Salmonella kentucky, 132
Salmonella lexington, 127
Salmonella london, 134
Salmonella manhattan, 132
Salmonella meleagridis, 132, 133, 137, 139
Salmonella minnesota, 132
Salmonella montevideo, 133, 134, 135, 138
Salmonella muenchen, 139
Salmonella new brunswick, 127, 139
Salmonella newington, 127, 137, 139
Salmonella newport, 132, 133, 134, 138
Salmonella oranienburg, 123, 132, 133, 134, 135, 138, 139
Salmonella oregon, 127
Salmonella panama, 134, 136, 139
Salmonella paratyphi, 120
Salmonella paratyphi A, 134
Salmonella paratyphi B, 134, 139
Salmonella pullorum, 135, 139, 140
Salmonella saint-paul, 123
Salmonella sandiego, 134
Salmonella schottmülleri, 120
Salmonella senftenberg, 134
Salmonella senegal, 123
Salmonella tennessee, 134, 135
Salmonella thompson, 133
Salmonella typhi-murium, 123, 127, 132, 133, 134, 135, 136, 137, 139, 140, 142
Salmonella urbana, 139
Salmonella worthington, 127, 132, 139
Sardines, 47
Sausage, 44, 47, 81, 86, 156, 157, 166
  canned Vienna, 146, 147
Sausage poisoning, 43
Scours, 167
Sewage, 155, 156, 160, 165
Shellfish poisoning, 8, 26–32
  dosage in, 28
  food concerned in, 8, 26, 27, 28
  incubation period of, 27
  outbreaks of, 26, 28
  poison in, 28, 29, 30–31
  sampling and testing in, 30, 31
  specific agent of, 27, 29
  symptoms of, 8, 27, 28, 29
  treatment of, 32
Shigella, 93
Shigella paradysenteriae, 163–64
Shrimp, 101, 133, 152
Snakeroot poisoning (trematol, milk sickness), 9, 26, 27, 35–36
  food concerned in, 9, 27, 35, 36
  history of, 36
  incubation period of, 9, 27
  specific agent of, 27, 35
  symptoms of, 9, 27, 36
Sodium bicarbonate; *see* Baking soda
Sourgrass, 46
Soybean mash (Natto), 46
Spinach, canned, 42, 45, 46, 59, 101, 132, 152
Spirochetal jaundice, 165
Sprats, 47
  canned, 67
Squash, 46
Staphylococci, general, 9, 98
Staphylococcus aureus, 91, 102, 106, 159
Staphylococcus food poisoning, 10, 33, 80–118, 122, 153, 157, 158, 161
  control of, 98–103
  enterotoxin, 104–16
    ammonium sulphate precipitation of, 114
    dialysis of, 115
    effect of proteolytic ferments on, 115
    mode of action of, 115–16
    production of, in synthetic mediums, 104–5
    solubility of, in alcohol, 114
  enterotoxin-producing staphylococcus, the agent in, 10, 103–4, 153
    effect of penicillin on, 107
  epidemiology of, 88–90
  foods involved in, 10, 87–89, 99, 101, 102
  history of, 80–88
  incubation period of, 10, 90, 92, 153
  laboratory diagnosis of, 92–98
  other toxins in, 97, 102–3, 111
  susceptibility to, 91–92, 94, 96
  symptoms of, 10, 90–92, 153
  treatment of, 92
Starch, 12
Streptococcus food poisoning, 11, 109, 146–54
  incubation period of, 11, 152, 153
  outbreaks of, 146–49, 150, 151
  Streptococcus faecalis, agent in, 146, 149, 150, 151, 152, 153
  symptoms of, 11, 147, 148, 149, 150, 151, 153

Succotash, 46
Sulphurous acid, 24

Tarts, 14, 88
  marmalade, 15
  treacle, 15
Tea, 16
Tetrodon fish, 32
Tin poisoning, 22–23
Tomato
  catsup, 46
  home-canned, 46, 48
  juice, 46, 133, 152
  relish, 46
Tongue, 88, 89, 107
  pickled, 47
Toxoids, 75
Trematol; *see* Snakeroot poisoning
Trembles, 36
Trichinella, 101
Trichinella spiralis, 166
Trichinosis, 11, 165–66
Tuna, 47
  creamed, 101
Turkey, 101
  dressing, 147, 149
Turnips, 46, 70
Typhoid fever, 132, 141, 155, 156, 162
Tyrotoxicon, 85

United States Department of Agriculture, 40, 43, 128
  Bureau of Animal Industry, 101, 128, 129
  Bureau of Chemistry and Soils, 22
United States Food and Drug Administration, 45
United States Public Health Service, 42

Vegetables, 46, 50
  frozen, 101
  home-canned, 10, 44, 46, 50
  for soup mixture, 46
Vicia fava, 27
Viral digestive upsets, 166–70
"Vitullary" fever, 85

Water, 14, 88
Water-hemlock poisoning, 9, 38–39
  food concerned in, 9, 27, 39
  incubation period of, 27, 39
  specific agent of, 27, 39
  symptoms of, 27, 39
Wheatcakes, 17
Wine, 16
World War I, 2, 9, 39, 42, 86
World War II, 3, 134
Wurstvergiftung, 43

Zinc poisoning, 14, 23
  dosage in, 23
  food concerned in, 14, 23
  sources of, 14, 23
  symptoms of, 14
Zinc sulphate, hydrated, 23

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